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ABSTRACT

A cross-disciplinary approach to the topic of sexual differentiation comprises this volume. Diverse papers are included under a variety of headings: 1) critical Periods in development; 2) embryology; 3) RNA-DNA; 4) chromosomes-growth and development; 5) physiology; 6) primates; 7) cognition; 8) cultural differences in patterns of sexual behavior; 9) normal and abnormal behavior; and 10) cross-cultural research on human behavior. The overall topic is seen as involving a consideration of molecules, man, and society with overtones of mysticism, religion, and social organization. The volume contains not only formal presentations, but also the discussion by the participants. (TL)

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ENVIRONMENTAL INFLUENCES ON GENETIC EXPRESSION:

Biological and Behavioral Aspects of Sexual Differentiation

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ENVIRONMENTAL INFLUENCES ON GENETIC EXPRESSION

Biological and Behavioral Aspects of Sexual Differentiation

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A SYMPOSIUM SPONSORED BY THE
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and Human Development
and the
John E. Fogarty International Center
for Advanced Study in the Health Sciences

National Institutes of Health
Bethesda, Maryland
April 22–25, 1969

Norman Kretchmer, M.D., Ph. D.
and
Dwain N. Walcher, M.D.
Scientific Editors

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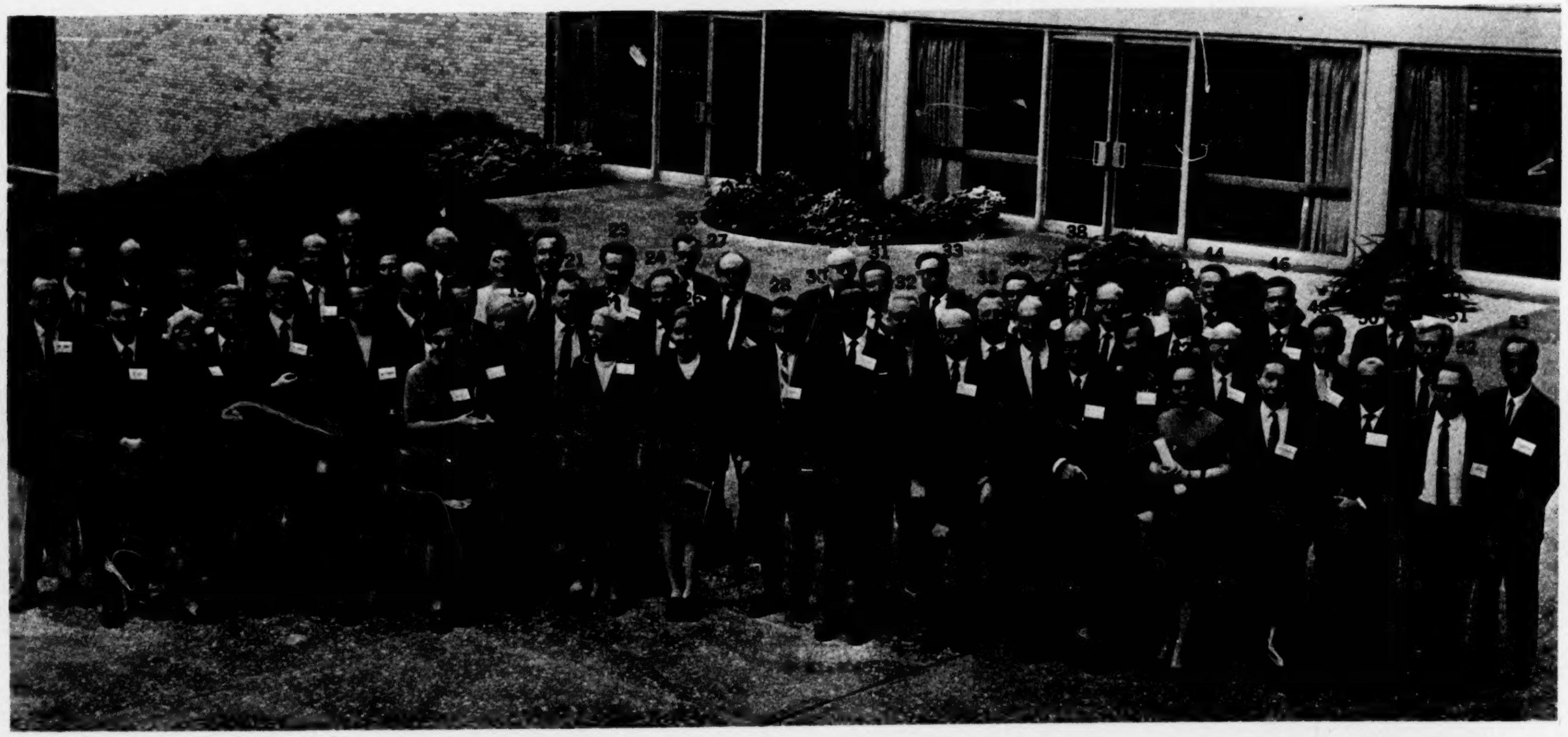

(1) Money, (2) Rapola, (3) Kagan, (4) Swinyard, (5) Barton, (6) Jacquot, (7) Jost, (8) Johnson. (9) Ambrose, (10) Caldeyro-Barcia, (11) Havighurst, (12) Papousek, (13) Prechtl, (14) Koldovsky, (15) Thomae, (16) Charalambidou, (17) Sereni, (18) Minturn, (19) Ehrhardt, (20) Hamburg, (21) Walcher, (22) Price, (23) Chapeville, (24) Hahn, (25) Hirschhorn, (26) Jennison, (27) Williams-Ashman, (28) Oliver, (29) Greulich, (30) Lambo, (31) Frenk, (32) Prader, (33) Garn, (34) Kretchmer, (35) Rossi, (36) Milkovic, (37) Barnett, (38) Richardson, (39) Feldman, (40) Jensen, (41) Baxter-Grillo, (42) Milkovic (Mrs.), (43) Condliffe, (44) Bongiovanni, (45) Woodside, (46) Tarkowski, (47) Nishimura, (48) Erdmenger, (49) Ribeiro, (50) Metzner, (51) Caudill, (52) Eto and (53) Sundaram.

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FOREWORD

The major purpose of this conference was to bring together a number of people from various scientific fields to consider a topic so broad that a cross-disciplinary approach to it could be effective. To this end, the planning group invited molecular biologists, embryologists, biologists, psychologists, sociologists, anthropologists, and clinicians to examine within a short period the topic of sexual differentiation, which involves the consideration of molecules, man, and society, with overtones of mysticism, religion, and social organization. The topic represents an area in which there is evidence of environmental influences on genetic expression and one in which an interaction of behavioral and biological disciplines can be readily demonstrated.

The ability to pass from concern with molecules, cells, and anatomical configurations to the holistic concept of the organism is indeed difficult. Molecular biology and other biological disciplines have approached their particular problems with a view to decreasing the variables and arriving at some concept of universal biological law. In contrast, the social science disciplines, instead of reducing variables, examine the many variables that emerge where the organism deals with his brothers and his entire environment. Both systems of thought have utilized the scientific method, but each has approached an examination of developmental phenomena in a different way, accumulating different kinds of data. There was no question in the minds of the conference planners that an examination of such an important topic as sexual differentiation would have to include both these approaches, for it is from both of them that new concepts in this complex area will come. We brought both together in the belief that the message of a concern with the organism must include both.

The conference was developed with the help of several organizations concerned with the necessity for cross-disciplinary examination of problems affecting man and his internal and external environments. Among them we would like to mention gratefully the National Institute of Child Health and Human Development, under whose auspices the conference was initiated, nurtured, and carried out; the John E. Fogarty International Center for Advanced Study in the Health Sciences, newly created within the National Institutes of Health, who supported the concept by providing transportation of participants from outside the United States and by financing the publication of this report; the Foundation for International Child Health and the Association for the Aid of Crippled Children, who contributed financial and moral support.

The International Organization for the Study of Human Development was the organizing, nongovernmental association chiefly involved in bringing together scientists from many places in the world. Individuals from all the continents of the world except Antarctica were present at the conference. In addition, students ranging from a freshman in college to postgraduates were invited from Finland, Greece, Guatemala, India, Japan, Nigeria, and the United States. The participation of young people in a conference such as this is a rare event, since most such conferences are limited to established members of the scientific community.

Of major significance among the accomplishments emanating from this conference was the cooperation of the four participating agencies, governmental and nongovernmental, mentioned above, all of which have different objectives but all share as a general goal the achievement of better understanding of human development. Representatives of these agencies, as well as conference participants, took part in a business meeting of the newly-formed International Organization for the Study of Human Development following the last session of the conference.

We believe that a number of the original objectives were accomplished in this conference. At the same time, we recognize that some were not quite reached. It is extremely difficult to meld the biological sciences with the behavioral sciences. The difficulty arises first from the differences in the stages of development of the sciences, and second from the language barriers among scientists—barriers growing out of differences in scientific and national languages and cultural backgrounds. These barriers were apparent in the initial stages of the conference. As the people gained a greater mutual understanding and an empathy for their scientific fellow, the separatism disappeared and academic camaraderie increased to the point where effective cross-disciplinary scientific conversation could be held. We learned, as others have in the past, that this rapport is stimulated not only by the mutual respect attained within the conference room, but also by sharing various social and cultural experiences. Several opportunities were provided during the period of the conference for participation in such events.

Scientifically, we were impressed by the whole group, the content and quality of material examined, and finally the ability of the participants to identify gaps existing in our knowledge in this field of human development: sexual differentiation. The conference has given to the scientific community a demonstration of the devotion and dedication of scientists from many nations and from several disciplines to the conception involved in the development of the human being.

Were we to conduct this conference a year or two years hence, we probably would narrow the topic, but we would choose the same types of scientists. We thank all of the participants for their efforts, understanding, and contributions.

This volume contains not only the formal presentations on the general topic, but also the discussion by the participating audience. There has been no attempt to report this discussion verbatim but rather to extract the essence and import conveyed by the discussants. We think the report should be of interest to scientists concerned with sexual differentiation. We hope that it demonstrates the ability of a diverse group to consider a subject to which all the members have made scientific contributions from various approaches.

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Stanford University
DWAIN N. WALCHER, M.D.
Pennsylvania State University
Scientific Editors

December 2, 1969

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Symposium on Environmental Influences on Genetic Expression held April 22-25, 1969

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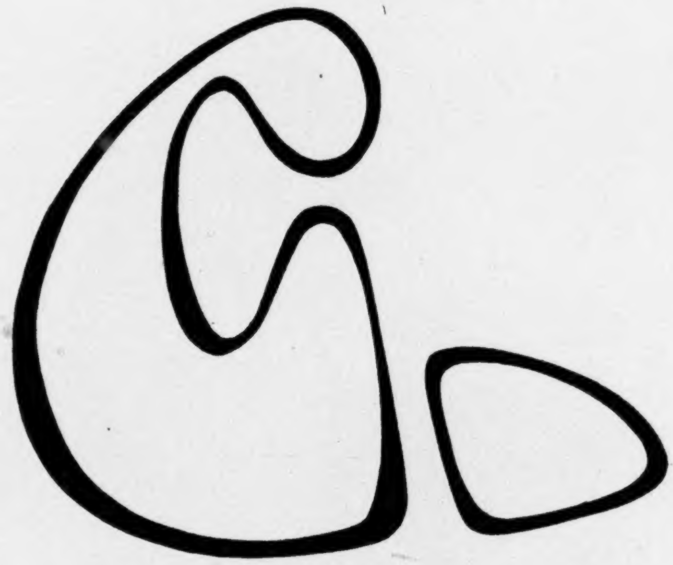
ENVIRONMENTAL INFLUENCES ON GENETIC EXPRESSION

BIOLOGICAL AND BEHAVIORAL ASPECTS OF SEXUAL DIFFERENTIATION



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THE INTERNATIONAL ORGANIZATION FOR
*THE STUDY OF HUMAN DEVELOPMENT**

**List of Founding Members (APPENDIX II)*

INTRODUCTION

At the first session of the symposium, Dr. Gerald D. La Veck, Director of the National Institute of Child Health and Human Development, welcomed the participants. "It is a particular privilege to open this conference," he said, "because of the close parallel of its purpose with the mission of the National Institute of Child Health and Human Development: the study of the entire spectrum of human development. We are indeed fortunate in having with us such an array of scientific talent as is represented by the distinguished scientists gathered here. I am confident that the exchange of ideas that will take place during the week will advance the study of human development.

"Under the heading of environmental influences on genetic expression, we shall discuss a wide range of topics. In doing this, we hope to lay the foundation for future conferences that will examine some of these questions in greater detail."

Because of recent scientific and political interest in the United States in two research issues, one dealing with the relation of malnutrition and intellectual development and the other with the possible modification of early childhood development through programs of intervention, the Institute is deeply involved in kinds of studies to which environmental influences on genetic expression are germane.

In the Winter 1968 issue of the *Harvard Education Review,* Arthur R. Jensen's paper on how much it is possible to raise I.Q. and scholastic achievement presents a scholarly view of the literature on intelligence, traces the history of the development of the psychometrics of intelligence, emphasizes the significance of the genetic component relative to the environmental component, and specifies certain physical and social environmental conditions that may affect the I.Q. of individuals and groups. He feels that the environmentalist view of intelligence has been detrimental to an understanding of its true nature, which he concludes is largely genetic.

The controversial element of Jensen's paper arises chiefly from his assertion that the basis of the observed fifteen-point Negro-White I.Q. differential is largely genetic in origin and from his opening statement that compensatory education has been tried and has failed. This article has received considerable attention in the lay press and will be discussed and argued for years to come.

Intelligence is an excellent example of environmental influences on genetic expression; it emphasizes the need for the development of the more precise delineation of the nature of environmental influences. Therefore, this conference, like many other endeavors of the NICHD, is an attempt to bridge the gap between the behavioral and biological sciences and between disciplines such as embryology and pediatrics on the one hand and those who study maturation and aging on the other.

"I am looking forward to a timely and successful conference," Dr. La Veck concluded.

Dr. Milo D. Leavitt, Jr., Director of the John E. Fogarty International Center for Advanced Study in the Health Sciences, greeted members of the symposium. He then spoke of the founding of the Center in 1968 as a division of the National Institutes of Health, named in honor of the late Representative Fogarty whose contributions to the advancement of the health sciences in the United States and abroad were substantial and far reaching. The chief objective of the Center, he said, is to advance and extend knowledge in the biomedical sciences through international collaboration, which involves close interaction among United States scientists and their colleagues in other countries. New programs at the Fogarty Center designed to meet this objective include the Scholars-in-Residence Program and the Conference and Seminar Program. These activities will involve bringing together American and foreign scientists at NIH for the development of science as it relates to health. The concerns of the scientists who will participate singly and in groups in these undertakings will range widely over issues related not only to maintaining the best characteristics and qualities of the human race, but to determining what those best characteristics are.

"The meeting that has convened here today is one example of the kind of thing we wish to stimulate at NIH. We are therefore delighted to be able to work together with the National Institute of Child Health and Human Development in the sponsorship of this symposium."

CRITICAL PERIODS IN DEVELOPMENT: INTRODUCTION

Clifford Grobstein
University of California School of Medicine
San Diego, California

In taking the broad-spectrum approach to human development indicated by the title "Critical Periods in Development," Dr. Grobstein noted that one places emphasis upon the fact that development is a progressive, time-dependent process. In large part, development represents the unfolding of genetic messages in particular environments.

Though one may attempt to separate the genetic message from the environment, for purposes of study, it is clear that heredity and environment cannot be truly separated in the consideration of any practical developmental problem. We can ask ourselves to what extent one or the other is involved in a particular aspect, but we cannot speak of a purely hereditary or purely environmental explanation of development.

In a global view of development, one is aware of a nonuniformity of environmental sensitivity in developmental processes; that is, there are varying degrees of stability and instability in the reaction of developing systems to environmental changes. This nonuniformity—the fact that there are times when the developmental process is particularly susceptible to the influence of environmental alteration or of given environmental factors—is the point we emphasize when we talk about critical periods in development. We are not, at the moment, clear about the underlying nature of such critical periods; it seems likely that the underlying nature is not the same in all instances. The existence of high sensitivity at particular times in developmental courses may reflect the nature of the particular developmental course; we may find different mechanisms contributing to this sensitivity in different developmental courses.

For example, it has been emphasized in Waddington's well-known spigenetic landscape (Waddington, C. H., 1940) that each of the decision points between alternative developmental courses may be a point of special sensitivity to environmental influences. Critical periods, in other words, may relate to choice.

But critical periods may also be found at the important points in development at which new levels of order are emerging. For example, in a developing cellular system the progression is from cellular to the multicellular state along with the progression from cellular homogeneity to heterogeneity. As inter-actions occur between different cell populations within the developing mass, tissue and organ properties emerge.

Certain kinds of critical instability in developing systems relate to the sensitivity of underlying processes themselves. In a trout hatchery the nurseryman observes a "tender stage" in the development of eggs: a short period during which great care must be taken to prevent any disturbance of the eggs. This stage comes at gastrulation, when the developing blastoderm is mechanically sensitive at its edge where it adheres to the yolk. A disturbance or change of temperature is likely to lead to separation of the blastoderm from the surrounding egg, frequently terminating development or producing abnormalities.

"Many such critical periods present maximum opportunity for intervention for control. It is the case, I think, that increasingly concern will turn to ways of controlling the developmental course. We shall attempt to accomplish this partly to make up for genetic defects and partly to try to improve development beyond the genetic blueprint. Either way, recognition of 'critical periods' and understanding their mechanism will prove useful to the developmental engineering of the future."

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CRITICAL PERIODS IN DEVELOPMENT

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THE PROBLEM

One day not so long ago, a physicist and a biologist left their laboratories and went to the mountains. They stopped to look at two mountains. One was a very high mountain and the other a very low one; one was brown, the other very green; one was rocky, the other smooth. The physicist said, "Look at those two *identical* mountains." Asked the biologist, "What is so identical about them?" and the physicist replied: "The *distance* between them is identical."

Though there may be some distance between scientists of the various disciplines here, because of the *identity* of this distance we may understand each other and agree on most of the points that may be raised in our discussions, with one exception. We may disagree about whether there is any point in discussing what I was requested to discuss: "critical *periods* in development." I personally have many doubts about it.

My main doubt is the following: If we first define a critical period as one without which further development will not take place, and start following fertilization, we find that the first morphogenetic event to take place is cleavage, cell division. If cleavage is inhibited, no further development will take place. The next dramatic event is gastrulation. If that is inhibited, no further development will take place. If neurolation—the formation of the brain—is inhibited, no further normal development will take place, and so on. In short, the entire process of development is a sequence of critical stages. If I were to discuss all of them, I would soon be facing empty chairs.

Let us return to that first example: a fertilized egg of a sea urchin manifesting the first critical stage of development, cleavage or cell division. Yet, there is a subcellular process that preceeds cell replication: a burst of protein synthesis, the first new molecular event that can be detected after fertilization. One may ask whether there is a causal relationship between these two events: Does protein synthesis in the fertilized egg determine cell replication? To answer this question, one can do a simple experiment: prevent protein synthesis after fertilization and see what happens. This has been done, and the answer was plain: if protein synthesis is prevented, no cell division will take place (Hultin, 1961). The same thing happens with the next stage, gastrulation: prevent protein synthesis prior to gastrulation, and gastrulation will not take place. In short, we see that different critical stages are determined by or are based on similar mechanisms or processes: protein synthesis. Therefore, instead of discussing the different critical periods in development individually, one can discuss the critical processes that determine the critical periods in development.

THE QUESTIONS

We all believe, for we have pretty good evidence for it, that the different critical stages in development are preceded and determined by the formation of different *types* of protein. This determines a second belief that has been formulated by Joshua Lederberg: "Make the polypeptide sequence at the right time and in the right amount, and organization will take care of itself," (Lederberg, 1966). How simple! But how do you make it in the right amount, at the right time, and in the right cell?

The really critical question, then, is how does each cell know when to make the right protein and how much of it to make? If one knows that, all the rest will follow.

Since all of us now are completely attached to the dogma that the different proteins are being coded by different genes, an immediate deduction invites itself: the developmental processes are triggered by the activation of different genes in different cells at different times of development. That, in fact, is the whole story. Then the actual question is how the different genes are triggered. Some people say that we do not have to worry about this; we already know how genes are being switched on. We know, because we have studied the famous bacterium *Escherichia coli*. "Everything that is true for the *coli* is true for the elephant," said Monod. This may be so, but let us look at two phenomena.

If you take a bacterium and switch on a gene, that gene—the DNA—will make m-RNA, and the RNA will automatically make the protein. The inhibition of m-RNA synthesis immediately inhibits protein synthesis. There is thus only one level of control in bacteria: an instruction to the gene. But is it true for developmental systems of multicellular organisms?

Let us go back to the fertilized egg of the sea urchin and inhibit the activity of the gene—prevent the DNA from making RNA. One can easily do this by applying a specific inhibitor for DNA primed RNA synthesis. But by doing that one does not inhibit the formation of proteins following fertilization. Proteins were found to be synthesized in the fertilized egg in the absence of RNA synthesis, that is, they are formed on a stored messenger RNA that has pre-existed in the ovum (Gross and Cousineau, 1963). Thus, in eggs there are two levels of control: the gene and the m-RNA.

A second example of a basic difference between control in bacteria and multicellular organisms is that the activation of genes in bacteria is a reversible process. You can instruct the gene to do what it should, and then you can stop it. If you give an inducer, say lactose, a specific enzyme, β-galactosidase is formed. You remove the lactose and enzyme synthesis stops.

The developmental processes are also triggered by the activation of genes, yet in most or very many known cases this is an *irreversible* process. Therefore, although it may be true that everything that holds for the *coli* holds for the elephant, not everything that is true for the elephant is true for the *coli*. And today, we are interested in the elephant, that is, in man.

I have said that different genes have to act in different cells at different stages of development. But, obviously, if they have to make this decision they must be ordered to do so; they must get some specific signals. We have known for more than 50 years that there are specific signals, specific inducers for brain development, for lens formation, for the development of all other organs. But if, indeed, there are specific inducers for activating different developmental processes, it is not enough to have an inducer: the signal must be recognized by cells if the cells are to be able to respond. To do so, the cells must have specific receptors for specific inducers. Without the concept of specific receptors, recognition cannot operate.

There are two types of signals that cell receptors need to recognize in developmental systems: one is free molecules, the other the cell-bound signals. Therefore, we ought to learn about the structure of the receptors that should recognize either free or cell-bound signals.

In many cases, the signals are macromolecules. If they are macromolecules, their capacity to penetrate cells is obviously very limited. If the capacity of the signals to penetrate cells is limited, then the cell receptors for recognition of specific inducers should be located on the cell surface. This again distinguishes our systems from those of bacteria.

If we assume that there are receptors and that a signal is being recognized, then what is the response to the receptor-signal interaction? There can be two kinds of response: the first is decision-making. A certain cell, in response to an inducer, decides to become something in a restricted sense, that is, it decides to restrict its capacity of gene activation to a certain spectrum of genes. For example, a mesenchymal cell is determined or committed to become, eventually, just a muscle cell, that is, it would be capable of activating only genes that code for proteins that determine muscle cell differentiation. It need not implement that decision immediately. For implementation, it may need a second signal. But its developmental fate is fixed and irreversible. There is no such thing in bacteria.

Not only is that state of decision completely irreversible, but it was found that even the progeny of the committed cell manifest that state. The molecular basis of this irreversibility is a critical question that cannot be answered by simply borrowing models from microorganisms.

The second response is the execution of the decision to become a certain kind of cell—say a muscle cell. This involves the expression of genes that code for muscle specific proteins. We then encounter the question of how the genes are activated at this point.

The question is far more complicated than in bacteria. For in many cases a cell must coordinate the activity of different genes located far from each other, which is unlike the case in bacteria, where the genes are located in operons and activated simultaneously. An example to illustrate this problem is this: our reticulocytes make hemoglobin composed of two types of polypeptide chains, the χ and the β chains. But the genes that code for these polypeptides are not closely linked to each other. In fact, they are on different chromosomes. Their activity must be coordinated. How is this done?

So far, I have referred to coordination of single cells. But the problem of growth is the problem of cell populations. Hence, we are faced with a different question. How many cells of a particular tissue does the organism make at any given time? At this very moment, new red blood cells are being produced within our bodies. Why do we not burst with progressive formation of red blood cells? Because the number of red blood cells that are formed at any given time equals the number of old red blood cells that die at that time. How does the organism know how to maintain this equilibrium?

EXPERIMENTAL APPROACHES

In our laboratory we have developed a few experimental systems that demonstrate how we can today approach *some* of the questions I have raised here. The first problem was recognition. If we assumed that there must be some receptors to achieve recognition and we want to identify them, then we must work, at least at first, with some defined signals or inducers. We know of many existing inducers that we can operate with. We can instruct the formation of brain, of lenses, of many other organs in frog embryos, but we do not know the chemical nature of any of these inducers. One inducer that I believe is a possible model for signal perception by cells in general is the *antigen.* Antigens are molecules that, when injected into the organism, elicit the production of specific antibodies. Different antigens elicit the production of different antibodies. Induction of an antibody response should then involve an initial phase of antigen recognition by cells. Recognition is believed to take place through a cell surface receptor. The existence of cell receptors for antigens was inferred from experiments in which free haptens inhibited the immunogenic effect of hapten-protein conjugates. Furthermore, closely related haptens interfered with the response induced by the hapten-protein conjugate; the antibodies inhibited were those that cross-reacted with the related haptenic determinant (Mitchison, 1967).

What is the nature of the cell receptors for antigens? It was suggested that the receptor may in fact be an antibody-like entity. This was derived from experiments in which antibodies inhibited the induction of a response, possibly by competing for the receptor for antigen. That immunoglobulins are present on the membranes of the lymphoid cell was supported by observations that antibodies to immunoglobulins induce transformation in lymphoid cells (Sell, 1967).

In our laboratory we conducted experiments based on the induction of antibody in organ culture. We applied as an antigen DNP (dinitrophenyl)-protein conjugates, and tested antibodies produced *in vitro* and directed toward the haptenic moiety, that is, the DNP. The first question we asked was: Are the cell receptors for antigen indeed antibody molecules located on the cell membrane? If the receptors are identical with the antigen binding sites of the antibody, then derivatives of DNP that bind covalently to anti-DNP antibodies should block irreversibly the cell receptors for DNP. These were produced by introducing a reactive bromoacetyl group into homologous derivatives of the DNP hapten. Thus, "affinity labeling" reagents, such as χ-N-bromoacetyl ε-N (2,4 dinitrophenyl)-lysine (BADL) and N-bromoacetyl N' (2,4-dinitrophenyl)-ethylenediamine (BADE), were prepared and found to bind covalently to anti-DNP antibodies.

The question then was whether BADL and BADE will form a covalent bond with the cell receptors for DNP, which will be manifested by a stable block of the receptors. We use the "Millipore" filter well technique for antibody formation *in vitro* which was developed by my colleague—Dr. A. Globerson—and Dr. R. Auerbach (1966). We adapted this system for the production of a primary immune response to DNP upon stimulation in culture with DNP-protein or DNP-polylysine conjugates (Segal, Globerson, Feldman, Haimovich, and Sela, 1970). This is the first case of an induction *in vitro* of antibody production to a chemically defined antigen. We then tested the effect of BADL and BADE on the immunogenic effect of DNP-hemocyanin and DNP-rabbit serum albumin. The result was that both BADL and BADE inhibit specifically the production of anti-DNP antibodies, and the effect, unlike that of free hapten-like DNP-lysine, persists for at least 44 hours (Segal, Globerson, Feldman, Haimovich, and Givol, 1969). In analogy with the affinity labeling of anti-DNP antibodies by BADL and BADE, we concluded that these reagents become covalently bound to antibody-like molecules on the cell surface of the spleen explants, which may represent the antigen recognition receptor of the antigen-reactive cells.

The recognition of specific antigenic determinants by the lymphocytes seems to me to be a model for any recognition system, such as recognition of hormones by target cells, of morphogenetic inducers, of growth stimulants. And the experimental approach to the characterization of the receptors for hormones, for embryonic inducers, and so forth, should be based on an experimental approach similar to the one I described for antigens.

Let us still remain within the antigen-cell system and ask what are the cellular events that are triggered as a result of the immunogenic recognition. It has long been known that following antigenic stimulation, the antibody-producing cells replicate exponentially and form clones of cells synthesizing antibody. A question, therefore, arises: Need the lymphocytes replicate exponentially in order to manifest the production of antibody? Need they replicate at all? Or is there perhaps a minimal requirement for replicating events that are essential for the activation of antibody production?

To test this, we again used the organ culture system for the *in vitro* induction of antibody to DNP. The first set of experiments tested the "secondary response" following the application of the antigen to spleen fragments from mice that had first been immunized *in vitro*. DNA synthesis was inhibited by applying cytosine arabinoside at various time intervals following the application of the antigen. Using DNP-hemocyanin as the immunogen, we found that if DNA synthesis was inhibited at time zero, that is, if the cytosine arabinoside was applied with the antigen, no antibodies were formed. But if the cytosine arabinoside was applied just 24 hours following the application of the antigen, an excellent production of antibody was recorded (Segal, Globerson, and Feldman). Considering the expected generation time of lymphocytes, these results were interpreted to indicate that a critical requirement for antibody production is one cycle of replication. Studies were then extended to other conjugates of DNP (that is, DNP-poly-L-lysine, and so forth) and to the process of primary response induced *in vitro* (that is, when the antigen is applied in culture to spleens of animals that have not been immunized with the antigen). An analysis was made of the minimal requirement of DNA replication, and the results were essentially similar to those found for DNP-hemocyanin, that is, that 24 hours of replication are sufficient to set off antibody production. With regard to some antigens and to the primary response, this critical period of replication starts 24 hours after the explantation of the spleen fragment and the application of the antigen. Yet, in all cases of DNP-conjugates tested in our organ culture system, a 24-hour time period of DNA replication was both necessary and sufficient. In other words, one cycle of DNA (or cell?) replication was essential and sufficient for the activation of antibody production (Segal, Globerson, and Feldman). How should one interpret this critical requirement?

For further consideration, let us turn here to the experiments carried out by A. Novik aimed at answering a similar question with regard to microorganisms. The induction of enzymes such as β-galactosidase in *E. coli* was usually attempted in bacteria that were at an exponential phase of replication. Yet, was this pattern of logarithmic growth of bacterial cells essential for enzyme induction? Novik tested this by inhibiting DNA and/or cell replication and found that the only requirement for enzyme induction was one cycle of DNA replication. This was attributed to the possibility that de-repression of a gene requires the production of a new strand of DNA, since on the "old" strand the repressor is firmly attached to the operator in a manner that is unsusceptible to change by the inducer molecule. Although one should be careful in extrapolating from the relatively simple microorganisms to the complicated multicellular systems, it is tempting to consider the possibility of a similar basis for gene de-repression, requiring the formation of a new strand of DNA, in our organisms. Gene de-repression may be achieved only on a newly formed DNA that has not yet been blocked by the repressor molecule.

I have discussed some critical processes associated with the response of cells to free molecular signals—in our case, the antigen.

Let us now consider the other type of signals generated by or carried on intact cells. To study this category, *in vitro* differentiation of muscle cells was investigated in our laboratory (Yaffe and Feldman, 1964, 1965; Yaffe, 1969). Rat myoblasts from trypsinized thigh muscles are seeded in culture. During the first few days they replicate; then on the fourth to fifth day, the mononucleated myoblasts line up along each other and fuse to form multinucleated fibers. Does the process of cell fusion involve recognition? Do myoblasts recognize each other? To test this, we mixed thigh myoblasts of two distinct genetic origins (rat and rabbit; rat and calf; rat and chicken), and tested the multinucleated fibers formed in such mixed cultures. The results were that hybrid fibers were formed with a random distribution of the nuclei from the two genotypes in each cell (Yaffe and Feldman, 1964). However, when thigh myoblasts were mixed with cardiac myoblasts of the same genetic origin, no fusion took place. As expected, neither did fusion take place when thigh myoblasts were mixed with kidney cells. It therefore appears that the myoblasts recognize each other as alike, across genetic barriers; they can distinguish myoblasts from heart or cells from other nonrelated organs. Yet, the mechanism of this intercellular recognition is still an open question.

The recognition of an immunogen by lymphocytes with specific receptors for that antigen triggers biosynthetic processes that end in the production of antibody. Does the recognition of myoblasts, leading to cell fusion, trigger biosynthetic events that underlie the morphogenetic differentiation of striated muscle cells? Tests were therefore made of the production of muscle-specific enzymes, such as phosphorylase, creatin phospho-kinase and myokinase, as a function of the fusion process. All these enzymes were found to appear, in fact to be synthesized, only after fusion (Yaffe, 1969). To test whether indeed fusion is essential for triggering the synthesis, attempts were made to prevent fusion, then follow the formation of muscle-specific enzymes. The removal of Ca^{++} from tissue culture medium was found to prevent fusion (Shainberg, Yagil, and Yaffe, 1969). Such cultures did not show enzyme activity. When, however, Ca^{++} was re-added, fusion of myoblasts took place, and the enzymes did appear (Shainberg, Yagil, and Yaffe, 1969). It thus appears that fusion does generate a signal for the synthesis of muscle-specific proteins. At what molecular level is the signal for enzyme synthesis received during the process of myoblast recognition and fusion?

I mentioned previously that, unlike the bacterial system, in animals the fertilization of eggs activates protein synthesis not at the level of the DNA, by gene de-repression, but rather by activating a preformed messenger-RNA. Is the activation of enzyme synthesis following myoblast fusion based on gene activation, namely on triggering transcription, or is it based on the activation of RNA, namely on triggering translation? Experiments were therefore made in which myoblasts were allowed to fuse under conditions of suppressed RNA synthesis, i.e., by treatment with actinomycin D. The result was that phosphorylase was synthesized following fusion under these conditions (Yaffe, 1969; Shainberg, Yagil, and Yaffe, to be published). It was therefore deduced that fusion signals the translation of m-RNA for phosphorylase that has pre-existed in the myoblast prior to the fusion process. Thus, the control of protein synthesis at the messenger level seems to be a widespread principle in mulicellular organisms.

Myoblasts are cells that are committed to form muscle fibers. The state of committment was most probably achieved by a specific inductive process. Yet, although committed, myoblasts require a second inducing signal in order to manifest their destiny—in this case, fusion. A similar situation, in principle, exists with regard to antibody production. Each lymphocyte appears to be committed to produce antibodies of one or a few specificities. The antigen activates the phenotypic expression of their commitment.

Can the state of commitment be inherited by the progeny of a committed cell? Studies conducted in our laboratory by Dr. D. Yaffe resulted in the selection of lines of myoblasts that replicated exponentially when passaged in culture and stored at low temperature while still retaining their capacity to differentiate (Yaffe, 1968). The molecular basis of the committed state is one of the greatest problems of cellular differentiation. The committed state is a critical state, the nature of which is utterly unknown.

I have tried to touch upon just some of the critical processes of cell differentiation. I have indicated to you the types of experimental systems that were developed in our laboratory for an experimental analysis of the molecular and cellular basis of these critical processes. Whether or not the optimism such systems seem to elicit today is justified remains to be seen.

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DISCUSSION

By translating "critical periods in development" into "critical processes," Dr. Grobstein said, Dr. Feldman has defined the problem more analytically. He also pointed to the importance of the genetic control of protein synthesis and the many puzzles still to be solved in attempts to understand it. His emphasis on these factors not only within cells but as they affect interactions between cells goes along with his stress on the need to concentrate on mechanisms peculiar to multicellular organisms. These mechanisms undoubtedly underlie many of our practical and social problems in development.

Dr. Feldman's observations on the reversibility and irreversibility of cell type, with his reference to conclusive demonstrations that a number of cell types are able to continue their type in culture, as in muscle, are of special interest, Dr. Grobstein continued. Yet it must be noted that there are other situations in which cells engaging in one specialized synthesis are able to give it up and undertake new ones. The pigmented cells of the amphibian iris can, in suitable circumstances, turn toward production of lens proteins, and the pigmented cells of the retina can turn toward formation of neural cells. The same thing is also spectacularly true in plant material, where we know that cells of specialized tissues can form entire new plant structures, with all of the cell types found in the full plant. As Dr. Feldman noted, the mechanism by which instructed cells are able to propagate their own types is of great interest; it must be coupled with the mechanism by which they are able to be reinstructed.

The critical step in many of these situations, as Dr. Feldman said, is the necessity for replication. Though the reason is not clear, and Dr. Feldman has not expressed dissatisfaction with any of the mechanisms proposed, he does suggest that it lies within the cell itself.

Dr. Grobstein asked whether investigations so far have entirely excluded the possibility that cells receive their information, at the time of replication, from their surroundings, conceivably from their own products. In all of the situations in which replication of type has been shown to occur, so far as he knew, it had taken place in multicellular aggregates, though they may have begun with a single cell. In such circumstances, the dividing cells are successively exposed to the neighbors they have themselves produced. Dr. Grobstein believed that the critical experiment of isolation of a single cell each time it divided and continued propagation in those circumstances had yet to be done.

Agreeing with Dr. Grobstein's formulation of the present state of affairs, Dr. Feldman thought that experimental situations that could lead to an answer to this question existed. It is now possible, he said, to induce formation of antibodies in suspensions of single cells as well as in tissue cultures. He was not sure, however, whether the replication required—the single cycle of replication—is a replication of the cell or of the DNA alone. In bacteria, it is possible to show that division of the cell can be prevented, just allowing replication of the DNA. This would be a sufficient condition for manifesting enzyme formation. But it is not known whether the same situation exists in multicellular systems.

Dr. McKhann asked Dr. Feldman whether he would extend his argument to say that once an extraordinarily stable cell, such as a neuron, gets beyond a certain stage of development it can be involved in the kind of process described. Dr. Feldman replied that he would not exclude the neurons, which, like muscle cells, have cells that do not replicate when, in fact, they start to manifest a state of differentiation. He considered the question open.

Dr. Rapola asked whether it was correct that mixing myoblasts from different animals—chicken and calf, for instance—produced a fusion at random. It would mean that there would be as many hybrid myotubules as non-hybrid, chicken and calf myotubules. If this is so, what happens with very unlike animals, such as newt and chicken? He asked whether Dr. Feldman had found any evidence that there would also be hybrid protein molecules if isozymes or such things were used.

With any two participants within the mammalian system, Dr. Feldman replied, the mixing of myoblasts of two different kinds of vertebrates will produce random fusion. If the procedure is carried out between a chicken and a mammal, quite a number of cells prefer to fuse with myoblasts of the same genetic origin. It may be that the farther apart the animals, the greater is the degree of genetic segregation manifested. Therefore, within the mammalian system there is random fusion, but across class boundaries the tendency is different.

Dr. Feldman said he could not answer the question about isozymes because he and his colleagues had not experimented with them.

This technique of cross-hybridization at the cell level, Dr. Grobstein observed, offers many opportunities for studying genetic expression and for developmental engineering.

Turning back to Dr. Feldman's definition of critical periods as periods without which no further development is possible, Dr. Hahn asked if this did not mean that there are innumerable critical periods, so that it would be difficult for anyone except those directly in the field to deal with the problem. Might one then say that a critical period is one during which certain processes can be affected by environmental factors more pronouncedly than at other times and hence direct further development, obviously on the genetic basis? This, as Dr. Feldman had indicated, becomes a conflict between geneticists and environmentalists. It has been believed that the environment is important at these critical periods because it can direct further development into one channel. This is the common ground between geneticists and environmentalists.

Dr. Feldman asked whether Dr. Hahn was talking about normal environment, not one that deviates much. Dr. Hahn replied that he was talking about nutrition, whereupon Dr. Feldman agreed. "But," he added, "if I had defined critical periods as you have suggested, I should have given a different talk altogether."

CRITICAL PERIODS IN ENDOCRINE EFFECTS DURING FETAL DEVELOPMENT

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During prenatal development, several hormones impose a change on other organs or physiological systems at definite stages. These events obey a fixed chronology, but they are suppressed by experimental removal or inactivation or by inborn absence or inactivity of the controlling gland. Examples are known in thyroid, sex, adrenocortical, and pituitary physiology, but the modalities and the significance for further life of the various hormonal stimuli acting on different organs are unequally well recognized so far.

I would like to survey schematically the characteristics of a few examples that were studied in our laboratory and that offer similarities and dissimilarities.

GLYCOGEN STORAGE IN THE LIVER OF RAT FETUSES

The quantity of glycogen in the fetal liver increases rapidly at a definite stage in development. In the rat fetus, this event occurs between days 18 and 19; it is controlled by adrenocortical hormones (Jacquot, 1959). It does not occur in fetuses deprived of corticosteroids (Fig. 1). (Deprivation of corticosteroids was produced by material adrenalectomy and fetal adrenalectomy or decapitation, the latter bringing about hypoplasia of the fetal adrenals).

Conversely, glycogen storage (Jost, 1966) and cytological maturation of the hepatocytes (Dupouy and Jost, 1969) can be anticipated on day 16 or 17 by giving cortisol to the fetus. It can also be postponed to day 21 in fetuses deprived of corticosteroids until day 20 and then given cortisol (Fig. 1).

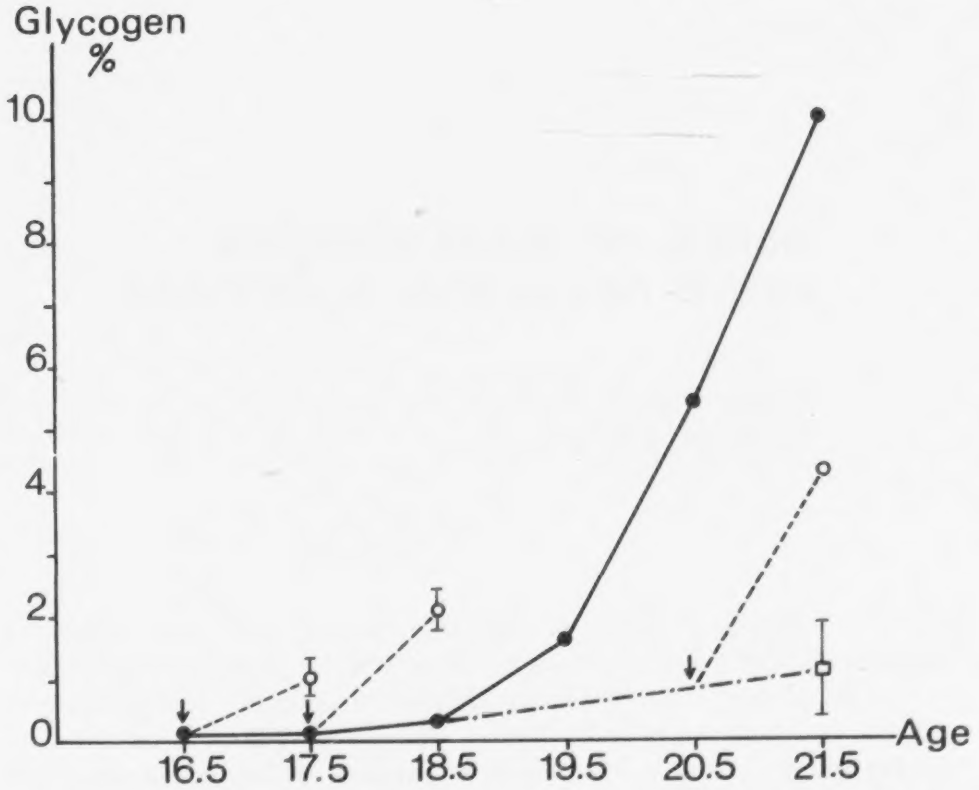

FIGURE 1.—Contents of glycogen in the liver of rat fetuses according to age in days and under various experimental conditions, (percentage of fresh tissue). Filled circles; controls of normal mother animals. Open square: fetuses decapitated on day 18.5 in adrenalectomized females. Open circles: fetuses injected 100 μg cortisol acetate (arrow) 24 hours before sacrifice.
Early glycogen storage in the entire fetuses given cortisol on days 16.5 or 17.5; delayed glycogen storage after a cortisol acetate injection on day 20 in decapitates of adrenalectomized females.

The shift in liver physiology, which normally occurs at a definite stage, can be hastened or delayed under experimental conditions, depending upon the presence or absence of corticosteroids.

GLYCOGEN STORAGE IN THE LIVER OF THE RABBIT FETUS

In rabbit fetuses, the glycogen stores in the liver increase rapidly after day 25. The hormonal control of glycogen deposition appears more complex than in rats, since two hormonal factors are probably involved: a pituitary hormone similar to growth hormone or to prolactin and corticosteroids (Jost, 1961). In rabbit fetuses decapitated before day 25, the liver glycogen remains low (Jost and Hatey, 1948;

Jost and Jacquot, 1955). It increases normally if the decapitated fetus is immediately given both prolactin and cortisone or cortisol, but not if it is given only the corticosteroid.

In fetuses decapitated on day 26 after the initial increase in glycogen storage has occurred, the rate of glycogen storage remains low during the next days, but it can now be elevated by cortisone given alone. After day 26, the corticosteroid treatment has become effective. If cortisone is given on the same day 26 to fetuses that had been decapitated three days earlier, that is, on day 23, no glycogen storage occurs (Jost, 1961, and unpublished data), (Fig. 2). It appears that the previous history of the system changes its reactivity to the same hormone.

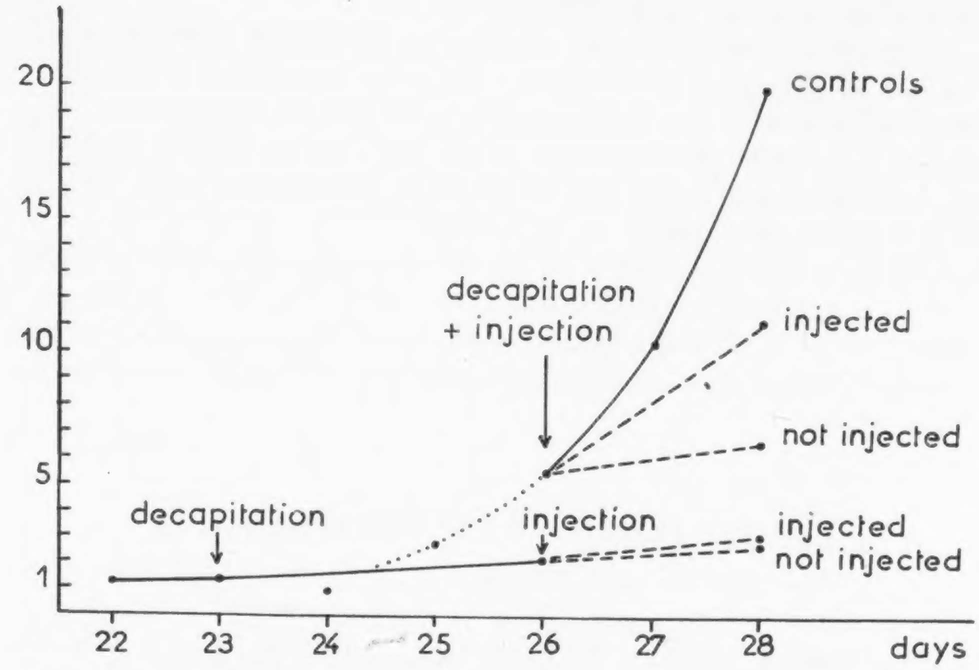

FIGURE 2.—Rabbit fetuses: glycogen content of the liver (milligrams per gram of fresh tissue) on day 28 in three series of fetuses: (1) controls; (2) fetuses decapitated on day 23 and injected (or not injected) with cortisone or cortisol acetate on day 26; (3) fetuses decapitated on day 26 and immediately injected (or not injected) with cortisone or cortisol acetate (from Jost, 1961).

Similarly, in fetuses decapitated on day 25, just before the increase in glycogen storage takes place, and given prolactin + cortisol, glycogen is stored during the next 24 hours; but the same hormonal treatment given on the same day to fetuses that had been decapitated two days before has no effect during the following 24 hours (Jost, unpublished).

Thus in the rat, corticosteroids are immediately active when given either on time or earlier or belatedly; in rabbits the result of the hormonal treatment depends on the previous presence or absence of the pituitary (in rats, the pituitary influence is probably replaced by the placental prolactin-like hormone).

Another point of interest in these experiments concerns the consequences of removing the pituitary during the period of rapid increase in glycogen storage, for instance on days 26 or 27 in rabbits; during the next days the amount of glycogen per gram of fresh tissue neither increases as in intact fetuses nor decreases to return to the low level found in the early-decapitated fetuses. It remains approximately at the level already obtained on the day of decapitation (Fig. 2). It appears that some benefit of the hormonal stimulus preceding decapitation is kept for several days. This intermediary level of functioning results from a "partial maturation" (Jost, 1968).

A similar intermediary level of function maintained after the interruption of the pituitary stimulus ("partial maturation") has been observed in several other fetal systems controlled by the pituitary. Examples are: weight of the adrenals in the rat fetus, activity of adrenalin synthesizing enzyme in the adrenal medulla, collection of iodine by the thyroid gland (Jost, 1968).

At present it is not known whether the incomplete maturation of these systems in the fetus has consequences for further life. Permanently perduring *partial* differentiation of the male external genitalia (hypospadias) results from interrupted or incomplete testicular activity during a limited critical phase.

CRITICAL PERIODS IN SEX DIFFERENTIATION

The study of sex differentiation of the mammalian fetus gives a good model of permanent effects of fetal hormones during critical periods, since it explains the irreversible realization of two different kinds of individuals from a common undifferentiated embryonic condition.

Castration experiments of rabbit fetuses were done at one-day intervals to explore the period of action of the testicular hormone on several structures (Jost, 1947; 1953a). Early castration (on day 19) prevents masculinization of the fetus; it suppresses the appearance of the prostatic lobes and permits the retrogression of the Wolffian ducts. After a late castration (day 24), the prostate is established and continues growing at a low rate, and the deferent ducts persist. Castration at successive intermediary stages shows successive stages beyond which different structures become permanently acquired. These stages are day 20 or 21 for the dorsal prostate, day 23 for the lateral prostate, day 24 for the deferent ducts.

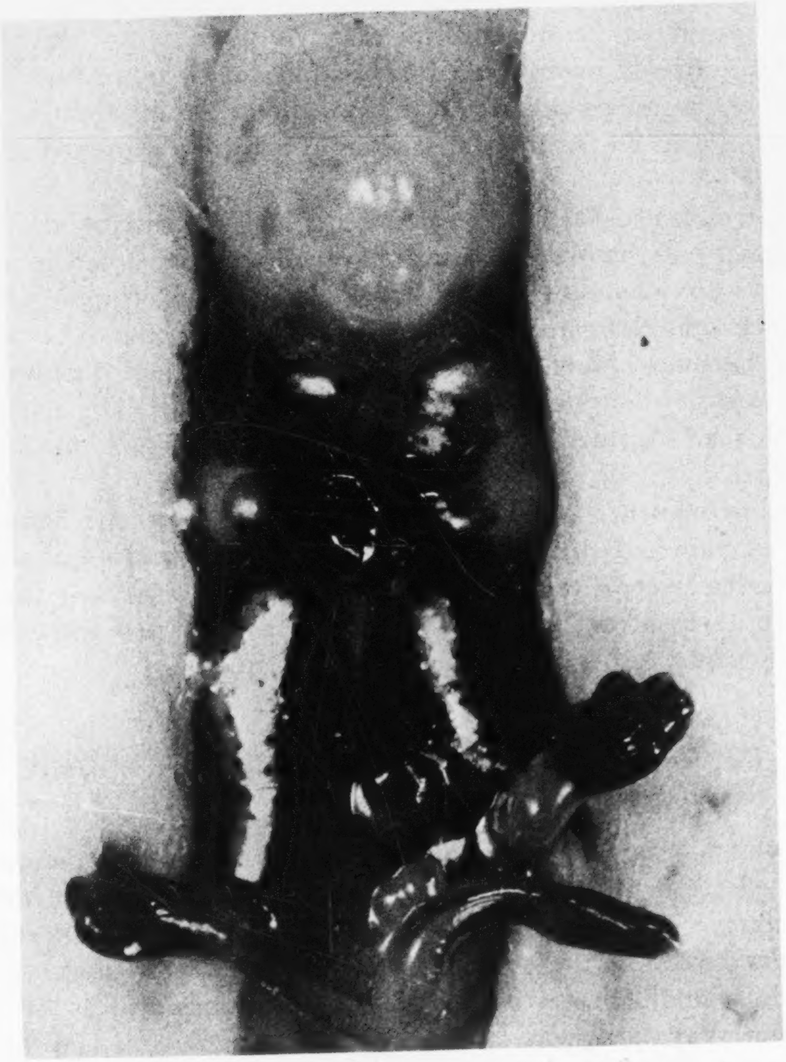

FIGURE 3.—Ventral view of a 18 day-old rat fetus injected intraperitoneously, 21 hours before, 250 mU of ADH: notice hemorrhagic swellings at the level of the limbs and tail.

These male sex characters develop in early-castrated males given testosterone. But testosterone either injected into the fetus or given to the mother cannot anticipate the appearance of the prostatic glands even by 24 hours in rabbits or in rats (Jost, 1947, 1961, 1967).

Male structures can be imposed in female fetuses by androgens given to their pregnant mother (review in Jost, 1969b). The exact period during which masculinization can be induced was studied in rats by giving a dose of androgen at various times during pregnancy and by studying the female fetuses 24 or 48 hours later. Prostatic development begins in males on day 18. It is easily obtained in female fetuses submitted to androgens during a period covering day 18. But it can neither be anticipated to day 17 by early androgenization nor be postponed by androgenization begun on or after day 19. Androgens can stimulate prostatic development on the urogenital sinus only during a limited critical period.

Other permanent effects of testicular hormones occurring during a critical phase were discovered: castration of young rats before the fourth day prevents the masculine type of hypothalamic gonadotrophic control (see Barraclough, 1966) and the masculine type of response to hormones of mating behavior (Grady, Phoenix, and Young, 1965). Testosterone injection into newborn females has the opposite effect. The testicular hormone has an organizing effect on some neural structure during the critical phase.

Another permanent neonatal effect of testicular hormone has been described recently by Denef and De Moor (1968) in steroid metabolizing enzymes in the liver of the rat. A female pattern of enzymatic activity develops in early-castrated males, unless they are injected with testosterone propionate.

CONGENITAL AMPUTATIONS AND OTHER ANOMALIES INDUCED BY HORMONES

Finally, another very different type of critical period in hormonal effects in the fetus should be mentioned. In rat or rabbit fetuses injected in the posterior with pituitary extracts or adrenalin, hemorrhages develop at the level of the extremities, that is, of the limbs, tail, tongue, mandible, and so forth. The hemorrhagic areas become necrotic, and as a consequence various parts of the extremity fall off or fail to develop (Jost, 1950, 1953, b,c; Jost, Roffi, et Courtat, 1969). Terminal lesions bring about congenital amputations; lateral or localized hemorrhages may produce club feet or other deformities (Jost, 1969a). Similar anomalies occur under genetic control in the *br* strain of rabbits (see Jost *et al.*, 1969).

The point of interest in the present discussion is that these lesions appear in the rat only if the fetuses are treated before day 18 (Jost, 1951, 1953c) and in the rabbit only if the fetuses are treated with adrenalin before day 23 (Jost, unpublished). It appears that these anomalies can be produced only during a critical period of development. The analysis of the hemodynamic events following the hormone administration suggests that the cause of these anomalies is a rather brutal transfer of blood from placenta into the fetus; this was measured with the help of labeled blood cells or albumin (Jost, Petter, Duval, Maltier, et Roffi, 1964; Jost, 1966).

According to this scheme, lesions are produced in the fetus only as long as the volume of blood passing from the placenta into the fetus is relatively important. Since fetal growth continues steadily until term, whereas placental growth ceases at a rather early stage, the relative volume of the placenta in the whole system decreases; therefore, anomalies of the extremities are no longer produced by hormones.

In the examples previously surveyed in this paper, critical periods in development resulted from biochemical events or from complex cellular differentiations occurring at definite developmental stages. In the case of limb anomalies, the time limit seems to be imposed by simple mechanical causes.

CONCLUSION

The very obvious morphological changes occurring during sex differentiation long ago permitted recognition of critical stages in testicular effects. It is now clear that permanent changes of tissues that take place during critical periods and that are either immediately apparent or not are much more frequent than was formerly suspected. The mechanisms involved in these changes and the modalities of their production are multifarious. The significance for further thriving of hormonal or environmental effects during critical developmental periods has become one important facet of the study of human development.

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DISCUSSION

Whereas Dr. Feldman had talked about the level of control within a cell—the control of protein synthesis, the direct impingement on that cell of influences from neighboring cells, and the consequence of these things upon the setting of the cell with respect to its specialized synthesis—Dr. Jost was describing phenomena of a much more complicated type, Dr. Grobstein observed. In dealing with early stages of development, stages in which cell behaviors are being changed, we now know something of the relationship between genetic and environmental aspects with respect to the cell. We are not able, however, to specify precisely the nature of the materials and mechanism by which the synthetic machinery within the cell is altered. On the other hand, in dealing with hormonal control we have reagents of great precision that we do understand, though we do not know how to translate the critical sensitivities into the critical underlying process.

Dr. Caldeyro-Barcia asked Dr. Jost whether he thought the failure to prevent growth of the prostate in his late castration of the rabbit fetus could be the result of the presence of other sources of androgens besides the testes at this time. In his injections of adrenalin and ADH, that had produced necrosis of the limbs, he had noticed vasoconstriction of the placenta, which would reduce blood flow to the placenta. Metabolic exchanges between mother and fetus would thereby be reduced, completely disturbing fetal homeostasis. How much would these effects contribute to the necrosis observed? It is interesting, he added, that injection of ADH acts only in a critical period that is well before birth. "On that system," interjected Dr. Jost, with which Dr. Caldeyro-Barcia concurred, since at the time of human birth there is a massive release of ADH. Apparently the human baby is resistant to ADH at that time. Why, Dr. Caldeyro-Barcia asked, does the necrosis happen only in the limbs?

Remarking that in his schematic presentation he had necessarily omitted many details, Dr. Jost said that the growth of the prostate was subnormal in rabbit fetuses castrated on day 24. But the prostate buds had appeared and grown. He referred to the precise determination made by L. J. Wells on castrated rat fetuses in this connection. *(Proc. Soc. Exp. Biol. Med.,* 63, 417, 1946.)

The possibility that some source of androgen is present in the fetus at later stages has often been discussed, but since the effect on the prostate depends upon the date of castration, and since the effects can easily be repaired by giving testosterone to the young animal, the indication is that there is not much testosterone and androgen hormones circulating in the system. It would have been impossible to obtain the castration effect at earlier stages if an androgenic substance had been circulating.

Dr. Jost and two of his students have been working on the effect of adrenalin on the placental and fetal vessels. In the placenta there is shrinkage of the fetal blood vessels; in the fetus superficial vasoconstriction in the skin, which becomes pale, is obvious. Many deep blood vessels become congested. In the limbs the large vessels are filled with blood and in the next hour or two after the injection hemoconcentration has occurred, fluid escapes into the connective tissue, and hemorrhages may occur. If the blood vessels do not rupture in such cases, the fetus recovers in the next six to ten hours. The reason why hemorrhages appear mainly at the level of the extremities is not definitely known.

Other people, Dr. Jost added, have observed the influences of adrenalin, etc., on placental circulation.

When one considers exchanges through the placenta, several hemodynamic factors are important. Acceleration or deceleration of the speed of blood flow is one of the factors that should be taken into consideration. Even if there are fewer blood vessels, or if the blood vessels are somewhat constricted, if blood circulates in a faster rhythm, the nutritional exchanges can be maintained to some degree.

In reply to Dr. Martini's question about whether the effect of the cortisone and prolactin is the same in operations on male and female rabbits, Dr. Jost said it was the same in either case. "But again," he added, "prolactin is not the only hormone that can influence the liver glycogen." "Then why," Dr. Martini asked, "did you choose prolactin?" "Because the rat placenta produces a lactogenic factor that probably works in the rat fetus, and because rat placental extracts are effective in the decapitated rabbit fetus. We used an ovine prolactin kindly supplied by the NIH. We also had a sample of ox growth hormone supplied by Dr. P. J. Randle, which was very active."

Asked about ACTH, Dr. Jost remarked that in an earlier research with Dr. R. Jacquot (see reference to Jost and Jacquot, 1955, in the text) he had got an effect in rabbits with a particular preparation of ACTH, but that hormone had also affected the genital tract and the thyroid; it was impure and possibly contained prolactin or growth hormone.

Dr. Martini also wished to know whether the implication in Dr. Jost's results with the castrated animals that did not develop a prostate might be that androgens were involved in the process. Is it possible that the same suppression of the development of the prostate might be obtained by giving antiandrogens instead of by castrating the animals? That Dr. W. Elger, Dr. F. Neumann, and Dr. Jost himself have published on the subject, was the reply. Cyproterone acetate prevents the effect of the testes on prostatic development and on the external genitalia in the rat fetus (Neumann, F., Elger, W., and Kramer, M., *Endocrinology,* 78, 628, 1966). But there are important differences according to the animal species. In rabbits cyproterone acetate prevents the formation of all the male structures (Elger, W., *Arch. Anat. Micro. Morphol. Exp.,* 55. 657, 1966; Jost, A., *Excerpta Med. Internat. Congr.* Series 132, 74, 1966); in the calf fetus a large dose has no effect (Jost, A., *Research on Steroids,* 3, 207, 1967). In the rat fetus cyproterone acetate prevents masculinization of the external genitalia and of the urogenital sinus, but even large doses do not suppress the Wolffian ducts and the formation of the seminal vesicles (Jost, A., 1967). The former structures are normally influenced by the testis on or after day 18, whereas the latter are influenced earlier, on days 16 and 17. Thus the antiandrogen prevents only those effects that occur on day 18 or thereafter. At least two hypotheses can be considered: 1) that there is a change in placental permeability to that compound, though Dr. Jost saw no proof for such a change; 2) that there is a change in the substance produced by the fetal testes at that time, and the early compound is not antagonized. The latter hypothesis would be very interesting, he thought, if it proved true.

Dr. Chapeville asked whether Dr. Jost had studied glycogen synthesis in the muscle, and other cells. If so, what happens, for instance, in muscle cells? Glycogen storage in the muscles, Dr. Jost replied, probably is not controlled to the same extent by hormones, but this has not been studied enough. He spoke of Dr. Kretchmer's studies of the effect of decapitating rabbit fetuses on the glycogen in the lung, which had resulted in finding no effect.

EMBRYOLOGY: INTRODUCTION

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University of Paris

If, in considering problems related to environmental influences on and genetic control of sexual differentiation during embryonic life, we look at the primordial germ cells and follow them after the formation of gametes, we find that at the morphological level, although different stages of development are well known, the mechanisms involved at these various stages are only partially understood.

Among the questions still to be answered are: What are the factors, at the chemical or molecular level, that control the migration of the primordial germ cells (apparently formed in the dermal layer) into the gonads? What are the potentialities of these primordial cells? What are the local influences in the gonad on the replication process of the cells when they switch from a mitotic to a meiotic type of division? Somatic cells of the gonad are involved in this process. How stable are the genetic factors controlling the course of gonadogenesis? Dr. Tarkowski will discuss some of these questions. Dr. Price will speak of the factors involved in sexual somatic differentiation and how androgens determine the differentiation of explanted cuts at the ambisexual stage isolated from males or females and retained in culture.

SEXUAL DIFFERENTIATION OF THE REPRODUCTION DUCTS OF FETAL GUINEA PIGS: AN EXAMPLE OF HORMONAL INFLUENCES ON GENETIC EXPRESSION

Dorothy Price
Professor Emeritus
University of Chicago

BACKGROUND

The relative importance of genetic factors and environmental influences on developmental patterns is frequently difficult to determine. The embryological development of the reproductive system of mammals offers a convenient model for an analysis. The clear-cut sexual dimorphism in sex-chromosome complement—XY or XX in males and females respectively—is normally correlated with clear-cut sexual dimorphism in morphogenesis of the reproductive systems in later stages of embryogenesis, but not in early ones. In early stages, there is an ambisexual phase during which the gonads are bipotential, every fetus has a double set of reproductive ducts (Wolffian and Müllerian), and bipotentiality is the rule in all other primordia of the reproductive systems. It is precisely the existence of the ambisexual stage followed by a sharp dichotomy in morphogenesis (depending upon whether the fetus is a sex-determined male or female) that provides an opportunity for analysis of environmental influences on genetic expression. The environmental influences that will be discussed in this paper will be fetal gonadal hormones, which have proved to be essential components of the internal environment.

The significance and importance of genetic factors in normal sex determination and differentiation have become increasingly clear in recent years from many studies of cases of spontaneously occurring abnormal sexual development in man (intersexuality, ambiguous sexual development, hermaphroditism) that could be correlated with abnormal complements of sex chromosomes or with sex-chromosome mosaicism. Cytogenetics has provided the tools for studies of karyotypes and sex-chromatin patterns. These techniques, as applied in man and in experimental work in the mouse, have enormously advanced the understanding of the relation of genetic factors, more particularly the importance of the Y and X chromosomes) to sex differentiation. Spontaneous abnormalties in sex differentiation occur in other mammals as well as in man, and these, too, are being studied.

The recognition that fetal gonadal hormones might normally play a decisive role in shaping the course of sex differentiation came from a study of a special type of intersexuality, that of the bovine freemartin (Lillie, 1916, 1917, 1923). The freemartin is a sex-determined female as deduced by Lillie in 1917 and corroborated forty years later by Moore and his associates (Moore *et al.*, 1957) in studies of the sex-chromatin pattern. The important factors in this now-famous case are that the freemartin is always co-twinned with a male, and as Lillie found, that there are anastomosed blood vessels in the chorions (which fuse at an early stage). From these observations, and from embryological studies, he proposed that fetal testicular hormones crossed to the female twin through the vascular anastomoses and caused masculinization. This intersexuality was interpreted as inhibition of the ovary and the Müllerian ducts by fetal testicular hormones, but stimulation of the Wolffian ducts and the formation of the male type of accessory reproductive glands.

Lillie's interpretation of the freemartin intersexuality was not as important per se as was his application of his interpretation to normal sex differentiation. He brought the problem clearly into focus by proposing that, normally, sex differentiation might be controlled by fetal gonadal hormones circulating in the blood stream. His evidence was limited to observable effects of testicular hormones (on the freemartin) and his theory was thus applied more particularly to sex differentiation in males. Specifically, he proposed that fetal testicular hormones are present by the end of the ambisexual stage in males and act as the morphogenetic agents that turn sex differentiation in the male direction by dual actions that are appropriately inhibiting or stimulating in responsive organs. Lillie stated that his evidence was limited and that he did not know whether fetal ovaries also secrete hormones that are responsible for normal female sex differentiation.

His theory was enunciated at a time when sex chromosomes had recently been identified in insects and the genetic mechanisms of sex determination and differentiation were being explored. Interest in the action of hormones was growing rapidly. Gonadal hormones had not yet been extracted, isolated, characterized as steroids, and synthesized, but this was soon to come. In this setting, the impact of Lillie's theory of hormonal influence on sex differentiation can only be estimated by the enormous volume of research (on all classes of vertebrates) that followed, and still continues to follow, his proposal of the hypothesis. Burns (1961) summarized and discussed much of the early research, including the results of administration of synthetic steroid hormones, results that were frequently confusing and paradoxical and shed little light on the factors that are involved in normal sex differentiation. Many techniques were used and much was learned, but no one reached the goal of producing an experimental freemartin (see Burns, 1961).

Mammals, in particular, proved difficult material for experimentation. The fetal environment, with the complication of the placenta, presents problems that require critical experiments and special techniques for solution. Extrapolation of findings in non-mammalian vertebrates to sex differentiation in mammals is tempting, but may not be completely justified. The hormonal theory of sex differentiation attempted to explain normal development on the basis of findings in an intersexual mammal—the freemartin—and may be summarized in simplified form. Male hormones from fetal testes of the co-twin:

1. masculinized the ovaries by inhibiting the development of cortical components and stimulating medullary components;
2. inhibited the Müllerian ducts (fated to form the oviducts, uterus, and part of the vagina in females);
3. stimulated the Wolffian ducts (which become the excurrent ducts of the reproductive tract in males);
4. stimulated the formation of male accessory glands (seminal vesicles).

The problem of the relation of genetic and hormonal factors in gonadogenesis will be treated elsewhere in this symposium. The experiments to be reported in the second part of this paper will deal only with sex differentiation of the reproductive ducts in relation to gonadal hormones, and a consideration of the evidence that these hormones modify genetic expression. The critical experiments for determining the relation of gonadal hormones to duct development (or retrogression) are those in which the morphogenesis of the ducts can be studied in the absence of the gonads. This was pointed out by Lillie in his early papers, but fetal gonadectomy *in vivo* is difficult in placental mammals, and almost thirty years passed before such experiments were successful. Then, almost simultaneously, fetal gonadectomies in the rabbit and in the rat were reported (see Jost, 1950; Wells, 1950). Their results showed that fetal testes in both species were secreting hormones (a question that was moot at that time but has long since been answered). The findings of Jost in the rabbit were more extensive and presented a clearer picture. They indicated that fetal testicular hormones maintain Wolffian ducts and inhibit Müllerian ducts in the male fetus of this species, and that sex differentiation in the female is not dependent on fetal ovarian hormones. For recent reviews of these and other experiments see Jost (1965), Price and Ortiz (1965), and Wells (1965).

Interesting and indeed unique as Jost's experiments on the rabbit were, a number of questions remained unanswered and corroboration and expansion of research in other species were essential. It seemed highly desirable to employ a method in which the effects of gonad removal on the reproductive ducts could be studied in the absence of possible influences from placental and maternal hormones. An organ culture technique was developed by which fetal mammalian reproductive tracts (complete except for the caudal region of the urogenital sinus) could be isolated and cultured under controlled conditions (Price and Pannabecker, 1956). The disadvantages of utilizing such an artificial system in studying sex differentiation are far over-balanced by the advantages. These include control of hormone content of the medium, the relative ease of replication of cases, the extraordinary degree of visibility of the ducts during culturing (allowing observation and photographic recording of development or retrogression) and, finally, the fact that excised tracts undergo a rather surprising normal type of sex differentiation when explanted and cultured under the system that we have used. This organ culture method was applied successfully in studies of sex differentiation in the rat (Price and Pannabecker, 1956, 1959) and in the mouse (Brewer, 1962; reviewed by Price and Ortiz, 1965).

The second part of this paper will present a summary of some of the recent organ culture experiments on guinea pigs that I have done in collaboration with J. J. P. Zaaijer, University of Leiden, and E. Ortiz, University of Puerto Rico at Rio Piedras. Much of this research has been published or is in press and all of it has been a collaborative project. In summarizing and discussing our studies, I shall focus attention on the following fundamental questions:

1. How early in development are testes of fetal guinea pigs secreting hormones, and how is this timing related to the period of sex differentiation of the ducts?
2. When do Wolffian ducts of males become dependent upon testicular hormones (male hormones, classified in more general usage as androgenic hormones) and retrogress in their absence?
3. When do Wolffian ducts of females become androgen-dependent, and are they as responsive to testicular hormones as their male homologues?
4. Is there evidence in these studies that Müllerian ducts of males are

normally inhibited by testicular hormones, and can Müllerian ducts of females be so inhibited?

A special point of interest for this symposium is whether homologous ducts (i.e., Wolffian ducts in males and females) become androgen-dependent at the same period and can respond to testicular androgens to the same degree although the sex-chromosome constitution of the cells is XY in one case and XX in the other. A second point is a more general one—an inquiry into the specific evidence that genetic expression is modified by fetal testicular hormones.

EXPERIMENTAL ANALYSIS OF SEX DIFFERENTIATION OF THE REPRODUCTIVE DUCTS OF EXPERIMENTAL GUINEA PIGS

Our general aim in this research has been to study the relation of the various stages in the process of sex differentiation (*in vivo* and *in vitro*) to secretory activity of the gonads, i.e., the presence of fetal gonadal hormones in the internal environment. To this end, we studied an extensive series of normal fetuses and prepared a chronological history of organogenesis in the reproductive tracts of males and females, devised a simple, effective "bioindicator" test for the presence of androgenic hormones in fetal organs, explanted male and female reproductive tracts at two critical developmental stages and cultured them under various conditions (presence or absence of sources of fetal androgens) and, finally, correlated the findings.

A. The organ culture method.—The technique that has been used has been described in detail (Zaaijer *et al.*, 1966). It is essentially a modified watch glass method using Falcon plastic culture dishes and a natural medium. An important point in the method is that the explanted organs or reproductive tracts are placed in position on a lens paper raft, which rests on top of the semisolid medium. The explants quickly adhere to it and flatten out. This flattening facilitates diffusion of food, oxygen, and waste products, greatly increases visibility of the ducts in the reproductive tracts, and simplifies transfers, which are made every two or three days during the culture period. The explanted reproductive tracts are never disturbed or dislodged from their original position at explantation.

At the end of the culturing period, the explants are fixed and prepared for histological study by routine techniques. Photographs and tracings are made during culturing to supplement observations.

The developmental history of the ducts can usually be followed clearly from the time of explantation to terminal fixation. Study of serial sections of the explants verifies our observations *in vitro* and provides the basis for detailed histological analysis.

B. Important stages in the embryogenesis of the reproductive tract in vivo.—The guinea pig is a long-term rodent with a gestation period of about 68 days on the average. However, the critical stages in sex differentiation are completed within the first half of gestation (by 37-38 days). To establish the timing of developmental stages in sex differentiation, 42 fetuses between the ages of 18 and 38 days were studied. This was later expanded by study of gonads and selected regions of the reproductive tracts in many older fetuses. Details have been presented in Price and Ortiz (1965), Ortiz *et al.* (1966), Price *et al.* (1967), and Price *et al.* (1968). Only a few important developmental steps are pertinent here.

1. Gonads: the gonads are demarcated from the mesial regions of the urogenital ridges at 22-23 days of fetal life, but at that time they are not distinguishable in routine histological preparations as testes or ovaries. By 24-25 days, medullary cords are forming in gonads that can then be identified as testes; by 26-27 days, testes are usually clearly identifiable and differentiation is proceeding rapidly. Ovaries remain undifferentiated until about 26-27 days, when faint medullary cords can usually be found, but at 30-31 days a typical ovarian cortex is forming.

2. Reproductive ducts: the Wolffian ducts are primitive nephric ducts that form in connection with the mesonephric kidney (to be supplanted by the definitive kidney—the metanephros). They are equally well developed in males and females up to 26-27 days, but by 29-30 days they are slender in females and degeneration (mainly cranio-caudal) is sometimes beginning. The processes of degeneration are fairly rapid and by 34-35 days only short solid cords remain in the caudal region; at 37-38 days, no remnants (except for small embryonic "rests") remain in females. In males, on the contrary, growth and differentiation with marked morphogenetic changes progress rapidly with formation of the primordia of the seminal vesicles by 30-31 days and coiling of the epididymal region by 34-35 days. It is apparent that the Wolffian ducts in males and females have ceased to belong to the nephric system by 26-27 days or soon thereafter (the metanephric kidneys with their ureters are then well formed), and these ducts are then adopted and adapted as part of the reproductive tract in males, but discarded in females—an old story in descriptive embryology.

The Müllerian (or paramesonephric) ducts begin their development at 22-23 days with the formation of ostia in the cranial region and grow caudally (at the same rate in males and females) in close association with the Wolffian ducts. By 26-27 days they have reached the urogenital sinus and are complete ducts, identical in the two sexes. However, by 29-30 days the cranial region in males is somewhat slender and degeneration then sets in, so that by 34-35 days only the caudal one-third remains; at 37-38 days, the Müllerian ducts of males are reduced to short remnants. In the female, growth and morphogenesis proceed rapidly; by 30-31 days there are indications of coiling in the oviductal region. This is marked by 34-35 days, and extensive at 37-38 days, when the oviduct is partially enclosed by the forming ovarian bursa.

It is clear that the period of 26-27 days marks the ambisexual stage in the reproductive ducts. Every fetus has a complete double set and no sex difference is apparent. The critical stage for retention or loss of ducts then follows.

C. Bioindicator test for the presence of androgens in fetal organs.—This test was first described in detail (Zaaijer *et al.*, 1966) in early studies of androgenicity in fetal organs (testes, ovaries, and adrenals) 30-36 days of age. It has now been extensively applied to younger and older stages. It involves culturing the organ to be tested with a small piece of ventral prostate gland from a young postnatal rat. The prostate is highly androgen-dependent and retrogresses rapidly in the absence of an androgenic source. *In vivo* postnatally, it is normally stimulated by testicular androgens, but it responds readily to endogenous androgenic hormones from ovaries and adrenals and to a fairly wide spectrum of exogenous androgens (for review see Price and Williams-Ashman, 1961). In our culture system, it retrogressed within 5 to 6 days (as in a castrated male *in vivo*) when cultured alone, and this period was selected for our test. Androgenicity, or its absence, was determined by the histological structure of the prostate explant. Approximately two hundred androgenic tests have been made on gonads, nearly one hundred on presumable nonandrogenic organs (mesonephros, metanephros etc.) and one hundred and twenty-five on prostate explants cultured alone (as controls with each experiment). Organs ranging in age from 22 to 62 days were tested. The results are:

testes	strongly androgenic from 22 days, when they are essentially undifferentiated and identification was made on the basis of development in culture, to 62 days, the oldest stage tested
ovaries	nonandrogenic from 22-36 days; weakly androgenic at 41-46 days; strongly androgenic at 60-62 days
mesonephros metanephros urinary bladder anterior pituitary	nonandrogenic
rat prostate	completely retrogressed when cultured alone for 5-6 days.

D. Explanted reproductive tracts.—The special modifications of our general techniques that were made for the explantation and culture of excised reproductive tracts were described with the first results of the work (Price *et al.,* 1967), and additional expriments have been reported (Ortiz *et al.,* 1967; Price *et al.,* 1968). The research that will be summarized briefly here deals with explantation of tracts at two specific periods: 26-27 days, when the reproductive ducts are completely ambisexual, and 29-30 days, which marks the beginning of sexual dimorphism *in vivo.* The important morphogenetic changes that occur during sex differentiation *in vivo* span approximately the period from 29 to 38 days, and our experiments were designed to span the same period *in vitro.* By 38 days *post coitum,* sex differentiation of the ducts has resulted in the dimorphism characteristic of males and females. Some of the results of culturing 37 tracts under various conditions of presence or absence of fetal gonadal hormones are summarized below, and the most important observations on the Wolffian ducts (WD) and Müllerian ducts (MD) are recorded.

1. *Male tracts explanted at 26-27 days;* cultured 9 or 11 days
   Testes present—WD retained and stimulated.
   Testes absent—WD completely retrogressed.
   Young ovaries replacing testes—WD completely retrogressed.
   Sixty-day-old ovaries replacing testes—WD retained and stimulated.
   Müllerian ducts retrogressed under all culture conditions.
2. *Male tracts explanted at 29-30 days;* cultured 9 days
   Testes present—WD retained and stimulated.
   Testes absent—MD partially (or completely) retrogressed.
   Young ovaries replacing testes—WD partially (or completely) retrogressed.
   Müllerian ducts retrogressed under all culture conditions.
3. *Female tracts explanted at 26-27 days;* cultured 9 or 11 days
   Ovaries present—MD retained; WD retrogressed.
   Ovaries absent—MD retained; WD retrogressed.
   Testes replacing ovaries—MD retained; WD retained and stimulated.
   Sixty-day-old ovaries replacing young ovaries—MD retained; WD retained and stimulated.
4. *Female tracts explanted at 29-30 days;* cultured 9 days
   Ovaries present—MD retained, coiling of oviductal region; WD completely retrogressed.
   Testes replacing ovaries—MD retained, coiling of oviductal region; WD variable.

E. Discussion and conclusions.—The results of these experiments on androgenic tests of gonads and on culture of explanted reproductive tracts have been discussed extensively elsewhere. Only a few points are of importance here. The finding that the testis is secreting androgens at the age of 22-23 days (when prospective testes cannot be distinguished from prospective ovaries in routine histological preparations) is noteworthy. This secretory activity precedes by almost a week the beginning of sex differentiation of the reproductive ducts as observed histologically. This timing suggests that testicular androgens must be available in the internal environment of the male fetus—by diffusion, or in the circulation, or by both means—by the end of the ambisexual stage at 26-27 days. This conclusion is fully substantiated by the results of explantation of fetal reproductive tracts at this stage. In those that were cultured without testes, retrogression of the Wolffian ducts of males occurred, and in a pattern similar to that of the Wolffian ducts of females *in vivo* (and, indeed, *in vitro*). When testes were cultured with female tracts, the Wolffian ducts were completely maintained.

There is no evidence in these findings that there is any differential response between the ducts in the two sexes. In both, the Wolffian ducts were androgen-dependent at the same stage and they were, apparently, equally responsive to testicular hormones. These results in the guinea pig confirm earlier experiments in organ cultures in the mouse (Brewer, 1962) but differ from observations in the rat (Price and Pannabecker, 1956, 1959; Pannabecker, 1957). The Wolffian ducts of fetal male rats were more responsive to testicular androgens (and to synthetic androgens put into the medium) than were those of females. In the fetal rat, then, an expression of the XY or XX ex-chromosome constitution in the cells, was apparent. In addition, it was found that Müllerian ducts in fetal female rats were more responsive to exogenous estrogens in the medium than were those of males. Such differences in responses to exogenous hormones in males and females have previously been reported (see Burns, 1961, for a discussion, more particularly on his studies on pouch-young opossums, which are equivalent to the fetuses of placental mammals).

The point that at 26-27 days the Wolffian ducts of fetal guinea pigs are androgen-dependent raises the question of when this critical period ends and the ducts are stabilized. The results of explanting tracts at 29-30 days showed that stabilization had almost occurred by that age. In some male tracts cultured without testes, the Wolffian ducts completely retrogressed, but in others, variable degrees of retrogression ensued. In short, these ducts had been almost completely stabilized by testicular androgens *in vivo* before explantation, and the critical period (during which testicular hormones are essential for stabilization) can be set as about four days.

In Brewer's organ culture experiments in mice, a much shorter and sharply delimited critical period for male Wolffian ducts was demonstrated. In similar experiments in the fetal rat, these ducts were still completely androgen-dependent and non-stabilized in male tracts that were explanted 3 days before term and cultured for 4-6 days.

It is interesting to compare these results with those of Jost (1950) on rabbit fetuses castrated *in utero*. When testes were removed at 19 days, Wolffian ducts retrogressed completely, but operations on succeeding days resulted in an amount of retrogression that was related to age. If gonadectomy was delayed until 24 days, stabilization had occurred.

In our experiments on the guinea pig fetus, the results on Wolffian ducts in female tracts explanted at 29-30 days illustrate another point. These ducts *in vivo* had been androgen-dependent, but had lacked stabilizing testicular androgenic hormones. Retrogressive processes had begun in the cells and had progressed so far in the Wolffian ducts of some females that testicular androgens, provided in culture, could not reverse or modify the course of degeneration. In explants from other females, testicular hormones were effective.

From these studies, a chronological history of the Wolffian ducts in fetal guinea pigs can be prepared and some conclusions drawn as to the role of hormones in modifying genetic expression. In the first phase, these ducts are nephric ducts and equally well developed in both sexes; this phase extends from 19 days (when the ducts have completed their caudal growth) to the ambisexual stage at 26-27 days. Their formation and growth is attributable to the genetic factors that govern the differentiation of the nephric system. The second phase involves an abrupt change in the cells from a hormone-independent state to one of androgen-dependency. Lacking androgens (normally provided in the male by testicular hormones), Wolffian ducts of males and females begin retrogression and the degenerative processes that start in the cells must be gene-controlled and linked to the normal retrogression of most of the tubules of the mesonephros. Gene-controlled, too, and exactly timed in ontogeny, is the development of androgen-receptor mechanisms in the cells in the ducts of both sexes. Androgen-responsiveness is dependent on this. The critical period in which androgenic hormones can, and must, modify the expression of genes that would cause the inception of degenerative processes lasts about four days. In this phase, we see a striking example of the modification of gene action by fetal testicular hormones. The final phase is one in which either the Wolffian ducts have been stabilized or the degenerative processes have advanced too far to be reversed and the ducts retrogress. Critical androgen-dependency (a matter of life or death) is over for stabilized Wolffian ducts, but they remain androgen-dependent for the development of completely normal structure and ultimate function. Genes and androgenic hormones must be visual-

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ized as in constant interplay beginning with the end of the nephric period of the Wolffian ducts.

The results of our experiments on differentiation of the guinea pig Müllerian ducts *in vitro* can be dealt with relatively briefly. In males, removal of the testes even at the ambisexual stage did not cause any retention of the Müllerian ducts—they retrogressed in the usual pattern. In females, the maintenance and development of the ducts did not depend upon hormones secreted by the ovaries, nor were the Müllerian ducts in females at all inhibited in the presence of testicular hormones (which, in many cases, stimulated the Wolffian ducts in the same explant). In earlier organ culture experiments in the rat, testis removal at even younger stages, before the ambisexual period, did not result in Müllerian duct retention. Again, testicular hormones did not inhibit these ducts. However, in Brewer's experiments in the mouse, Müllerian ducts were retained or partially retained if testes were removed during a short, critical period early in development. The findings on explants supplied with testicular hormones were in complete agreement with the findings in the guinea pig and rat—no retrogression of the Müllerian ducts. Weniger (1964) used a different method of organ culture and reported that Müllerian ducts in fragments of male mouse tracts were retained if testes were absent, and that Müllerian ducts in fragments of female tracts that were explanted with testes were retained.

Jost (1950, 1953, 1955) observed complete retention of Müllerian ducts in fetal male rabbits that were castrated *in vivo* at an early critical stage. Castration at later stages was less effective. In addition, there were two reported instances of unilateral retrogression (that was partial) when a fetal testis graft was introduced into the coelom of a female and placed against one side of the reproductive tract. There was ample evidence in experiments on gonadectomy of females that the Müllerian ducts were not dependent on ovarian-secreted hormones.

The observation that the guinea pig testis is secreting androgenic hormones at early stages in development raises the fascinating question of the character of this secretion. It is beyond the scope of this paper to discuss the evidence that testes may be secreting several hormones and that different ones stimulate Wolffian ducts and inhibit Müllerian ducts. Our findings in the guinea pig indicated that the testis was secreting androgenic hormones, and recent observations suggest that these androgens may be steroidal. Black (1967) made an electron microscopical study of testes of fetal guinea pigs 23-24 days old and found, in the interstitial cells, areas of smooth endoplasmic reticulum. These conspicuous areas of angranular endoplasmic reticulum were similar to those found in the interstitial cells of testes of adult guinea pigs (Christensen, 1965) and proposed, from several lines of evidence, as the site of biosynthesis of androgens. More recently Black and Christensen (1969) reported smooth endoplasmic reticulum in interstitial cells and in presumptive Sertoli cells of 22-24-day-old testes of fetal guinea pigs, and the relation of such reticulum to the production of steroid hormones was emphasized.

Bloch (1967), in studying guinea pig testes at mid-gestation, found that they were capable of steroidogenesis. However, this capability is probably developed much earlier, An added piece of evidence comes from recent unpublished observations of Verhoef-Bouwknegt and Zaaijer. They demonstrated the enzyme 3β-hydroxysteroid dehydrogenase in the gonads in urogenital ridges of 23 and 24½-day-old guinea pigs when the contralateral gonad was definitely identified as a testis by differentiation in culture and gave a positive prostate bioindicator test. Previous studies (reviewed by Price and Ortiz, 1965) had shown the presence of enzyme activity at older stages. In the capability for steroidogenesis and in the possession of 3β-hydroxysteroid dehydrogenase the fetal guinea pig testis falls in line with those of many other mammalian species (for reviews see Price and Ortiz, 1965; Bloch, 1967; Jost, 1967).

A CURRENT VIEW OF THE PROBLEM OF MAMMALIAN SEX DIFFERENTIATION

In the first section of this paper, the hormonal theory of sex differentiation in mammals was presented in the classic form in which Lillie proposed it fifty years ago. It is certainly time to reconsider the problems, and biologists in the field of fetal endocrine physiology and embryology, genetics, cytogenetics, and biochemistry are doing just that. We must question how much of the hormonal theory has been substantiated and still stands (although sometimes in modified form). We must view the freemartin itself with critical eyes and question whether the masculinization of the reproductive system is entirely attributable to fetal testicular hormones from the male twin.

In the realm of gonadogenesis, the relation of sex-determining factors to the processes of sex differentiation needs and awaits clarification. The role of somatic and germinal constituents, of early secretion of testicular hormones, and of corticomedullary inductor systems (see Burns, 1961, for a review of Witschi's long-standing theory) must be considered in the light of findings in cytogenetics and genetics. A very promising approach to the problem has been made in ingenious experiments on fusing mouse eggs of different genotypes, transferring the resultant blastocyst to a pseudopregnant female for development, and studying the progeny (Tarkowski, 1965; Mintz, 1965, 1968; Mystkowska and Tarkowski, 1968). Some of these are hermaphrodites (all are cellular mosaics), and the analyses of the results are contributing importantly, not only to an understanding of the effects of mosaicism but also to an understanding of the factors in normal differentiation.

As for the ducts and accessory glands, the major tenets of the old hormonal theory of sex differentiation have been fully substantiated. Fetal testicular hormones are, indeed, essential to maintain the Wolffian ducts of males at the end of the ambisexual stage, and they will do so in females under experimental conditions. Some of the supporting evidence was reported in the previous section. In addition, the role of testicular androgens in the formation of the male type of accessory reproductive glands has been verified (see Price and Ortiz, 1965).

Evidence for the inhibiting action of fetal testicular hormones on Müllerian ducts is limited to experiments *in vivo* in the rabbit and *in vitro* in mice, and further study is essential. In particular, the evidence for inhibition of Müllerian ducts of females is slight.

In the demonstrations that testicular hormones act on the cells of Wolffian ducts to prevent genetically-controlled retrogressive processes and thus stabilize the ducts, the pathways by which this is accomplished remain an open question. And, similarly, the way in which testicular hormones (the same or perhaps quite different ones) cause cellular degeneration and death in the cells of Müllerian ducts (apparently stable ducts) is equally unknown. The receptor mechanisms that insure hormone-responsiveness in the cells present inviting problems for biochemists and are the subject of active investigation.

And what of the freemartin as a model of the action of fetal testicular hormones on a sex-determined female? It has been known for about twenty years that almost all bovine twins have identical blood groups. This would be most unlikely unless primitive blood cells were transported from one partner to the other in the blood that is exchanged. It has been demonstrated that these twins are, indeed, red cell chimeras (erythrocyte mosaics). Furthermore, in almost all cases, skin grafts made between adults are accepted. Somatic cells, then, are exchanged between twins, but what about germ cells (typically migratory in early stages in fetuses)? Ohno and his collaborators (1962) have now added to the picture that germ cells can and do cross in the conjoined blood streams. The freemartin, already known to be a mosaic in some respects, may prove to be a germ cell chimera as well. Just how this may be linked to the inhibited ovaries and Müllerian ducts and the stimulated Wolffian ducts and accessory glands remains to be shown. The important point is that the early studies on the freemartin resulted in a theory of normal sex differentiation that has, in part, been substantiated and has been provocative and stimulating. The freemartin has not been discarded as a scientific "has-been," but has become the center of fresh interest and new scientific work.

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DISCUSSION

Both Dr. Price and Dr. Jost had shown that external male genitalia and the structures of the Wolffian duct are androgen-dependent, Dr. Prader noted. One would, then, expect that the new intersexes in females, if they were exposed to an abnormal androgen influence during fetal life, would develop and differentiate the structures of the Wolffian ducts. But in clinical experience this does not happen. In the adrenal-genital syndrome the female develops external male genitalia, but no Wolffian ducts. When fetuses have been exposed to external androgens—either androgens given to the mother or when the mother has an androgen-producing tumor—the external genitalia remain, but the Wolffian ducts are not present, not differentiated. This human experience leads one to conclude that the external genitalia depend on androgens, but that the differentiation of the Wolffian ducts does not; or perhaps the latter requires a much higher concentration.

"The crux of the situation," Dr. Price replied, "is that we have controlled experience. We know when the androgens are being introduced in relation to the critical periods. This is not the case with human beings. In human babies after birth, one sees the end of the story. There is no way to know when those androgens were first available to the wolffiettes."

Dr. Garn had pointed out, Dr. Prader added, that the timing is very important. This might be the explanation in the case of the adrenal-genital syndrome. It could be argued that the abnormal production of androgens starts too late. But there are in the literature examples of mothers who had androgen-producing tumors from the beginning of the pregnancy, or even before, and received testosterone early in the pregnancy. With these mothers the exposure to androgen was early enough to cause the Wolffian ducts to differentiate, if they are really androgen-dependent.

The point is, said Dr. Price, that even in such cases one cannot be sure when the androgens reach the Wolffian ducts *in effective amounts.* Exogenous androgens are quite another matter. The human placenta is a fascinating object. Effective amounts of the hormone must be present at the critical period when the Wolffian ducts can respond. Also there must be responsive Wolffian ducts in this testicular feminization, more appropriately called androgen insensitivity. Some rats have ducts that do not have the right clues—do not recognize the hormones.

Addressing both Dr. Price and Dr. Jost, Dr. Bongiovanni asked whether he was correct in thinking that in the intact animal, such as the rabbit, giving large doses of testosterone at any period in the pregnancy would not change the internal structure of the female fetus.

Dr. Price said that she had not worked with rabbits. She doubted that one would have any trouble maintaining Wolffian ducts with exogenous hormones in the female rabbit.

"The maintenance of Wolffian ducts can easily be achieved with several kinds of androgenic compounds," Dr. Jost replied, "but usually one needs much more hormone to maintain Wolffian ducts than to masculinize external genitalia." He cited Sherman's work with rats, in which a dose of one milligram of testosterone for primates given during the last eight days of pregnancy produced masculinization of external genitalia and suppression of the vagina, but only exceptionally resulted in maintenance of the Wolffian ducts. It was necessary to increase the dosage to 2.5 milligrams a day to maintain the ducts.

For lack of time, Dr. Price noted, she had not taken up the thresholds of response of the various parts of the reproductive tract.

"I feel sure that there is real danger in projecting from one species to another," Dr. Price added. "Interpreting what is happening in the human being on the basis of Dr. Lillie's observations in the freemartin is entirely fallacious. Dr. Lillie said that his observations on the freemartin were intended as an interpretation of sex differentiation of the male in the freemartin. They might have wider application, but he felt they would not do for interpretation of all intersexes. I think that if he were alive today, he would not be at all disturbed by finding out that there is a possibility that the freemartin may be a germ cell chimera. In rats and mice there is no trouble at all about obtaining Wolffian ducts that are complete by introducing exogenous hormones. The Müllerian duct is another story. But one should recognize that each species may be a law unto itself.

"The process of sexual differentiation does not stop at the Wolffian ducts," remarked Dr. Levine. "Some of us believe that the central nervous system is also involved. It is possible to alter sex differentiation for a period long after internal and external genitalia have been masculinized. By depriving the system of androgen long after these systems are well established, one can get a reproductive system that is feminine in all its functional reproductive aspects."

Dr. Price referred to the pioneer work of the late Dr. William Young in injecting androgens into pregnant guinea pigs, inducing not only intersexuality in the females but also female mating behavior in different strains of guinea pigs that did not have it. Her own strain of guinea pigs, she said, did not know what their sex was. Everything was all mixed up when they reproduced.

"The point is," she continued, "that the critical period for the androgen to get into the pregnant female to modify the central nervous system's organ for male mating behavior was just between 30 and 35 days. Dr. Young fortunately heard, just before he died, the results of our first experiments in which we were able to show that definitely testicular androgens were normally present *in vivo* in the male we are talking about—indeed, must be present—and that there was a good chance that the male mating behavior then was imposed on the central nervous system centers that mediate such behavior."

Dr. Hirschhorn brought forth a point in his work on XO/XY mosaics in human beings that is relevant to the current discussion. "A number of these individuals," he said, "have a hormonally normal testis with epididymis and Wolffian structures on one side, but also having undifferentiated ovarian tissue with strictly Müllerian structures bilaterally. The external genitalia can be strongly masculinized.

"The reason why there are no Wolffian structures on the side where there is no testis is obviously that the amount of inducing 'hormone' being secreted is in high concentration only very locally; it therefore maintains the Wolffian structures on that side. On the other side, there is regression of the Wolffian structures.

"On the other hand, the resorption of the Müllerian structures in such intersexes apparently requires the presence of two normal testes—a situation that occurs in a number of the human intersexes. Unless there are two testes, the Müllerian structures remain. Wolffian structures, however, respond to the local development of a gonad on one side or the other or on both sides."

The unilateral maintenance where there is a gonad that is primarily testicular or ovarian is common among mammals, Dr. Price observed. It may be due to local diffusion. Both Dr. Wells and Dr. Jost have postulated and demonstrated this possibility. As for all of the hormone that reaches the various parts of the tract in cultures by diffusion, it should be remembered that organs are typically leaky, which provides a good place to find diffusion effect. She thought that when hormones are first secreted they diffuse to begin with, and circulate later. These are very complicated situations without a simple explanation, however.

Dr. Price also suggested that in cases having ovotestes on one side and mainly testicular tissue on the other, it may not be clear that those ducts are sex mosaics, though very likely they are. The response of those ducts will then depend upon the proportion of XX or XY cells in them.

ARE THE GENETIC FACTORS CONTROLLING SEXUAL DIFFERENTIATION OF SOMATIC AND GERMINAL TISSUES OF A MAMMALIAN GONAD STABLE OR LABILE?

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INTRODUCTION

Over the past two decades, there has been accumulation of conclusive evidence that in mammals sex differentiation of the gonads precedes and determines sex differentiation of the originally ambisexual reproductive tract (for reviews see Jost, 1965; Price and Ortiz, 1965; Price, this symposium). While the gonadal primordium of a female embryo appears not to be involved in somatic sexual differentiation, the embryonic testes are actively engaged in this process. Differentiation of the sex ducts and the accessory structures in a male embryo can be explained in terms of stimulation and/or inhibition by substances diffusing between the interacting tissues, the gonadal tissue being the active component of this system. Thus, in searching for the first causative agent responsible for sexual differentiation, one has inevitably to turn to the gonad.

Two lines of research have contributed most to the understanding of the factors involved in the differentiation of a mammalian gonad. First, investigations on sexual differentiation in human beings and other mammals displaying aberrant karyotypes and second, studies concerned with the influence of adult and embryonic sex hormones on the development of the gonadal primordium. The results of these studies lead to the following conclusions.

First, the Y chromosome is necessary for testicular differentiation and consequently for the development of the male phenotype. Although this conclusion is supported by considerable evidence, there are also exceptions to the rule, e.g., some examples of human true hermaphrodites and of Klinefelter syndrome, which have a female (XX) karyotype. However, Ferguson-Smith (1966) recently formulated a hypothesis that such individuals carry Y chromosome material translocated to one X chromosome. The problem is probably more complicated than it looked at first, and presence of autosomal genes modifiers is also postulated to account for some exceptional cases (see Hamerton, 1968).

Second, the type of sexual differentiation of a genetic female, at both the level of the gonad and of the whole reproductive system, can be regarded as a neutral, so to speak "recessive," type of differentiation.

Third, at the gonadal level the intrinsic sex-determining factors are very stable. This conclusion is supported by the fact that attempts to reverse sex differentiation of gonadal primordia with sex hormones have been unsuccessful in placental mammals. On the other hand, there is evidence that differentiation of embryonic ovaries is affected, if they develop in a direct contact with embryonic testes. A limited degree of masculinization has been observed by several authors, the most convincing and best documented studies being probably those by Macintyre (1956).

Notwithstanding all the qualifications referred to above, the statement that the genital ridge develops into a testis or into an ovary, depending on whether or not the component cells contain the Y chromosome, is a tremendous step forward. However, it is but a first step. A task of developmental biology is to investigate the chain of events between the chromosomal or gene level and the tissue level. To express the point emphatically, the clue to the understanding of the phenomenon of sex lies in discovering how the genetic factors are translated into a definite type of differentiation—both morphological and physiological—of the gonadal primordium.

One of the possible ways of approaching this problem is to study differentiation of somatic and germinal tissues of the gonads in which cells of both genetic sexes have coexisted from the very beginning of embryonic development. One may hope that such studies will help to clarify the extent to which the genetic determination of differentiation of these two components—each of different origin and different fate—is stable and the extent to which it can be modified by environmental factors.

SOMATIC TISSUE

In a rather "cruel" experiment, nature occasionally creates individuals composed concurrently of cells of both genetic sexes. The etiology of this condition is not uniform: some individuals originate because of mitotic error in the development of a genetically male embryo and are designated mosaics; others result from incorporation into an embryo of cells derived from two zygotes of unlike sex and are designated chimeras. The record of spontaneous sex chromosome mosaics and chimeric mammals, including human beings, is already immense and is continually increasing (for references to XX/XY chimeras, see Tarkowski, in press). The majority of individuals displaying this abnormal condition are found because of visible sexual disturbances. This fact might create the faulty impression that sexual disorders, including true hermaphroditism, must inevitably develop in such individuals, and that the cases recorded represent the real extent of sex chromosome chimerism and mosaicism in the general population. The results of experiments described below show that in the mouse, at least, sex chromosome chimerism is compatible with the development of normal male and female phenotype, and that the development of true hermaphroditism is an exception rather than a rule. Since this subject was recently thoroughly reviewed (Tarkowski, in press), it will be treated in this report only briefly.

Fusion of cleaving embryos is so far the most efficient method of producing chimeric individuals in mammals (for technical details, see Tarkowski, 1961, 1965; Mintz, 1962, 1964; Mystkowska and Tarkowski, 1968, and in press). On *a priori* grounds, 50 percent of all embryo fusions should be between genetically male and female embryos, and one might expect that sexual disturbances, mainly true hermaphroditism, will be very common among chimeric animals. However, this was not found to be true. In the first two series of experiments carried out by the present author and his collaborators (Tarkowski, 1961, 1963; Mystkowska and Tarkowski, 1968), the sex ratio was predominantly male and the incidence of hermaphrodites was below the expected level. In view of the discrepancy between the expected and the observed frequencies of sex phenotypes, it was suggsted (Tarkowski,1961) that some sex chromosome chimeras are "hidden" among phenotypically normal males, thus distorting the sex ratio. The assumption that chromosome chimeras need not interfere with the development of a normal male phenotype has now received karyological confirmation. Two adult and fully fertile CBA-p/CBA-T6T6 males (Mystkowska and Tarkowski, 1968) as well as four male embryos (Mystkowska and Tarkowski, in press) proved to be sex chromosome chimeras. The only hermaphrodite available for chromosomal examination turned out also, as might have been expected, to be a sex chromosome chimera. Sex chromosome chimerism was also observed by Mintz (1968) in a hermaphrodite, sterile female and both sterile and fertile males.

In our recent studies in which embryos used for fusion originated from an albino outbred colony, the A inbred strain, and from the cross between A/CBAF hybrids and A animals (Mystkowska and Tarkowski, in press), the preponderance of males was less accentuated (about 60 percent), and in a sample of 40 animals, hermaphrodites were absent altogether. Exceptionally rare occurrence of hermaphrodites and a nearly 1:1 sex ratio were also observed by Mintz (1968). The latter observation is of special interest, but it does not invalidate other data, even if those refer to smaller samples. It seems that pooling data from many series, differing as to the combination of genotypes used, can conceal and obliterate the real differences among them. And these are the differences that may be illuminating.

One point in these studies is open to criticism and must be discussed. Chimerism in general, and sex chromosome chimerism in particular, are being estimated in these animals on the basis of examination of nongonadal tissues. To what extent can we estimate the composition of the gonadal tissue from the observed numbers of genetically male and female cells in the bone marrow, the liver, or any other somatic tissue? It seems that such an extrapolation is warranted; according to our experience, the majority of animals obtained by fusion of embryos display fine-grained chimerism all over the body, as far at least as it can be judged by pigmentation markers (Tarkowski, 1963, 1964a; Mystkowska and Tarkowski, 1968). On the other hand, evidence presented by Mintz (1965a and b, 1967) seems to indicate a much lower incidence of animals displaying two-color pigmentation. It is, however, very likely that, at least in a substantial number of sex chromosome chimeric individuals, the somatic tissue of the gonads is composed of both XX and XY cells. This view is substantiated by the fact that, at least in two cases of spontaneous origin, XX and XY cells have been observed in testes of adult individuals (Overzier, 1964—man; Malouf *et al.*, 1967—cat). There is thus suggestive evidence that, when XX cells are intermingled in the genital ridge with XY cells, they are subordinated to the latter cells and contribute to the testicular tissue. Whether these cells are physiologically neutral, "foreign stones" in the framework of a testis, or whether their physiology has been modified to the extent that they become involved in hormonal activity typical for the opposite sex is a completely different question, which at present cannot be answered.

This susceptibility of XX cells to the influence of XY cells could be tentatively accounted for by the fact that at the initial stages of sexual differentiation the somatic tissue of the presumptive ovary appears to be, in comparison with the tissue of the presumptive testis, physiologically "immature" and inert. Studies of Coulombre and Russel (1954) and Mintz (1959, 1960) on the W mutation, and investigations of Bennett (1956) on the Steel mutation in the mouse have shown that in the male embryos the genital ridges devoid of primordial germ cells undergo more or less normal histogenesis; sex cords are formed. On the contrary, in a mutant (W) sterile ovary, the somatic tissue does not display any specific type of histological differentiation, and the ovary retains the character of an undifferentiated gonad. It follows that, while in the male genital ridge primordial germ cells are not necessary for testicular differentiation, in the ovary these cells are actively engaged in organizing the somatic tissue and in stimulating its further differentiation. Once the primitive follicles are formed, the more or less homogeneous tissue differentiates into at least two types of cells—follicular cells and stromal mesenchymal cells. Another line of evidence, based on bioindicator tests and enzymatic studies, indicates the early commencement of hormonal activity by a fetal testis and the absence of such an activity in the ovaries (see Price and Ortiz, 1965; Price, this symposium).

In view of the facts that hermaphrodites can occur among chimeras and that ovotestes rather than testes or ovaries are occasionally formed, dominance of XY over XX cells should not be overemphasized. It seems that canalization of differentiation of genetically female cells into testicular tissue requires in the first instance a high degree of intermixing of cells of both genetic sexes, and that large territories composed of XX cells resist masculinizing stimuli, even if those are exerted at the very appearance of the gonadal primordium. Another qualification has resulted from studies made by Mintz (1968), and also by Mystkowska and Tarkowski (in press), showing that sex chromosome chimerism is also compatible with the development of a normal female phenotype. This conclusion can be indirectly inferred from the fact that in our last series and in the whole sample of Mintz (1968) the sex ratio approximates 1:1. Using karyological methods, we have so far identified two sex chromosome chimeric females; in both, XY cells were in the minority. There is no doubt, however, that in some combinations of genotypes male rather than female sexual differentiation of a sex chromosome chimeric embryo is a more common event. Further studies using various genotype and various genetic combinations of the two chimeric components are clearly necessary, as it may be that in the sexual differentiation of these animals, factors more subtle than those concerned with chromosomal sex of cells are also involved.

GERMINAL TISSUE

Does the course of gametogenesis in mammals depend on the genetic factors intrinsic to the germ cells, or on the environmental factors, i.e., on the phenotype of the gonadal tissue in which these cells reside?

After a period of gonial division, which follows their entrance into the genital ridge, the history of the mouse primordial germ cells becomes different in each sex. In the ovary, all germ cells enter into meiotic prophase (Brambell, 1927; Mintz, 1959; Borum, 1961). In the testis, at the corresponding stage, mitotic activity of germ cells slows down and gonial division become exceptionally rare. The coincidence in time of these two phenomena seems significant. Inhibition of gonial divisions in testes lasts till the first days after birth, when massive degeneration of germ cells takes place and the remaining cells become definite spermatogonia (detailed observations were made only on the rat: Clermont and Perey, 1957; Beaumont and Mandle, 1963; in the mouse the picture is very similar: Mintz, 1960). According to unpublished observations in this laboratory, spermatocytes in meiotic prophase can be seen in the male mouse for the first time about the tenth day of postnatal life.

Both genetically male and female germ cells are coded for meiosis, but they start it at different stages of ontogenesis, and after completing meiotic prophase they follow different routes of differentiation. Why germ cells enter into meiosis in general and why those in the ovary begin this process before birth is completely unknown; no sound hypothesis has yet been advanced. Let us confine the discussion to the ovary. There are two alternatives to account for the initiation of meiosis: first, it is induced by somatic ovarian tissue at a certain stage of its differentiation; second, it is due to some intrinsic factors in XX germ cells themselves that start to operate at a certain stage of their life history. Is, then, the physiological clock of genetically male cells different from that of the female germ cells? Why do they not enter into meiotic prophase at the same time as the germ cells in the ovary?

Experiments carried out recently in our laboratory (Ozdzenski, in preparation) have shown that when genital ridges from gentically male embryos are transplanted at a very early stage, their differentiation is slightly disturbed and a number of germ cells enter into meiotic prophase at the time schedule characteristic for ovaries. It seems, therefore, that like XX germ cells, XY cells can initiate meiosis as early as XX germ cells during embryogenesis, and that they can do this even in the testicular tissue composed exclusively of cells of the male genetic sex. It would follow that in normal development of a male this process is prevented or inhibited by some unknown factors, which most probably originate in the somatic tissue. The inhibition of gonial divisions, which is observed at this time, might be regarded as an indication that the conditions prevailing in the testis are unfavorable in general for initiation of prophase, either mitotic or meiotic.

Let us consider the problem from the opposite end. Can the mammalian germ cells of one sex form gametes characteristic for the opposite sex? Observations of Mystkowska and Tarkowski (1968) and of Mintz (1968) on artificially produced mouse chimeras are illuminating. Mystkowska and Tarkowski tested seven adult CBA-p/CBA-T6T6 chimeric animals for the presence of gametes of each genotype. The two chimeric components could be distinguished from each other by means of pigmentation and chromosomal markers. It was found that while the animals built of like-sexed components produced two genetic types of gametes, in the three sex chromosome chimeric animals (two fertile males, one hermaphrodite) spermatozoa were produced exclusively from the genetically male component, even though the X cells participated to a high degree in the somatic tissues. Moreover, XX cells were not represented among primary spermatocytes in diakinesis. Mintz (1968) also believes that in chimeric animals gametes are always derived from the component whose genetic sex corresponds to the phenotypic sex, and consequently that the course of gametogenesis cannot be reversed. It is noteworthy that in a XO/XYY mosaic male mouse, Evans *et al.* (in press), discovered spermatogonia and primary spermatocytes derived from XYY components only. As we did, Evans and his colleagues consider the absence of genetically female germ cells (in this particular case, XO) as secondary rather than primary.

Contrary to these observations, evidence has been presented that, in sex chromosome chimeric bulls and male marmosets, XX primordial germ cells, which—as it is postulated—have migrated to the genital ridge via vascular anastomoses from the female co-twin, persist in testes as spermatogonia and can even reach the first meiotic metaphase. However, since these observations have not been confirmed by other authors, their judgment should await further confirmation (for references and discussion, see Tarkowski, in press). A separate observation by Kjessler (1966) of XO spermatocytes in diakinesis-metaphase I in two XO/XY human mosaics also awaits confirmation. It would be very desirable if chromosomal examination of such individuals was, whenever possible, extended to the germ cells.

There is thus suggestive evidence that in the mouse, at least, genetically female germ cells are not able to complete spermatogenesis. When are these cells eliminated from the testicular tissue? To clarify this matter, studies have been undertaken on late embryonic and early postnatal chimeric males (Mystkowska and Tarkowski, in press). In five out of eight 16- and 17-day male embryos germ cells in meiotic prophase were encountered in the otherwise normal testes; three of these five individuals were karyologically examined, and all proved to have XX and XY cells in their livers. Although it would be tempting to postulate that the cells in meiotic prophase are the cells of XX constitution that realize their developmental potential irrespective of the atypical environment, such a conclusion does not seem to us to be true. The following three observations speak against this assumption. First, the observation by Ozdzenski referred to above, showing that XY germ cells can initiate meiosis as early as during embryogenesis; second, disagreement between the ratio of XX to XY cells in the liver, and the ratio of meiotic germ cells to prespermatogonia in the testis, the incidence of meiotic germ cells being as a rule much lower than the incidence of genetically female cells in the liver (Mystkowska and Tarkowski, in press); third, differences in the incidence of meiotic germ cells in the two testes of the same individual.

While these observations do not exclude the possibility that the meiotic germ cells are of XX constitution, they favor the alternative interpretation, namely that the initiation of meiosis is not indicative of genetic sex of a given cell and consequently that these cells can equally well be genetically male or female. It would follow that the initiation of meiosis reflects existence within a testis of a chimera of different microenvironments. In some parts of the testis, the local environment resembles more than the environment prevailing in the ovary and consequently meiosis is "permitted," while in other parts it is not "permitted." This conclusion is to some extent supported by the fact that cells in meiotic prophase usually occupy large sectors instead of being scattered singly or in small groups all over the gonad. In places where clusters of meiotic cells are seen, sex cords are often not well delimited, thus suggesting that the testicular differentiation was slightly affected. It has to be admitted, however, that meiotic germ cells often coexist at the same levels of sex cords with typical prespermatogonia. This slightly complicates the picture, as it renders necessary an assumption that small differences in the physiological state ("clock") of germ cells at the critical phase of somatic differentiation of a gonad are of importance, and that only the cells that entered into meiosis early enough, i.e., when it was still "permitted," are represented among meiotic germ cells.

The existence within a testis of a sex chromosome chimera, of a variety of local microenvironments, is quite likely, although the evidence is only indirect. As was discussed before (p. 55), we are not in possession of strict quantitative data regarding the participation of XX and XY cells in the somatic gonadal tissue and their spatial distribution within the gonad. If we accept the view that somatic gonadal tissue, like nongonadal organs, displays a fine-grained chimerism, then we can postulate that, although XX cells contribute to the typically testicular structures without disturbing histogenesis, they do not act physiologically, at least at this early stage, in the same way as do XY cells.

The results of studies aimed at discovering the further fate of germ cells that entered into meiosis before birth can be summarized as follows. 1) In one case (5-day-old CBA-p/CBA-T6T6 chimeric male) growing oocytes were found in the sex cords of a normally built testis (Mystkowska and Tarkowski, 1968). The genetic sex of these cells remains unknown. 2) None of the twelve chimeric males aged eight to twenty days had oocytes in the sex cords (Mystkowska and Tarkowski, in press). It seems, therefore, that formation of oocytes is a very rare event, and that the majority of germ cells that began meiosis prenatally degenerate before entering into growth phase or shortly afterwards. This conclusion is substantiated by observations made by Ozdzenski (in preparation) showing that, although initiation of meiosis in the transplanted genital ridges of male embryos is not uncommon, formation of growing oocytes is a rare event.

If it is accepted that prenatal initiation of meiosis in testes of chimeric embryos results from environmental rather than genetic factors, and taking into account the fact that meiotic germ cells constitute usually only a small fraction of the total germ cell population, the question arises as to the fate of the remaining XX primordial germ cells. One possibility, which seems to us rather unlikely, is that they degenerate during the gonial phase and do not succeed in reaching meiotic prophase at all. The alternative is that some XX germ cells persist in the testis, acquiring cytological characteristics of normal prespermatogonia. As already mentioned, however, it is known that, in sexually mature males, cells of this genetic sex are not represented either among spermatozoa or among primary spermatocytes in diakinesis (Mystkowska and Tarkowski, 1968). They may, therefore, be eliminated either shortly after birth, when spermatogonia are formed, or they may persist among the spermatogonia for longer times, until they degenerate during meiotic prophase. Attempts have been undertaken in our laboratory to find out which of these two alternatives, if any, is correct.

Next to nothing is known about the fate of XY germ cells in the ovarian tissue. In view of the fact that XY germ cells are able to enter into meiotic prophase prenatally and even occasionally to form growing oocytes (Ozdzenski, in preparation), and that in the ovarian tissue of the new-born chimeric hermaphrodites all germ cells initiated meiosis (Tarkowski, 1964b), it seems likely that XY germ cells placed in the ovarian environment enter into meiosis at the same time as XX cells. However, their further fate remains unknown. Although this is not explicitly stated in the paper by Mintz (1968), it seems that this author has not observed formation of ova from XY cells.

CONCLUSIONS

Taking into account the limited number of observations, we can formulate the following provisional conclusions:

1. Genetic factors controlling differentiation of XX somatic gonadal tissue are comparatively labile—XX cells intermingled within the genital

ridge with XY cells contribute to the formation of testicular tissue. A high degree of intermixing of cells of both genetic sexes appears necessary, however: large populations of genetically female cells resist masculinizing stimuli and form ovarian tissue. It has been shown also that sex chromosome chimeras can develop into females; but it remains unknown whether the somatic ovarian tissue of these animals contains XY cells.

2. The time of initiation of meiosis is under environmental control. Initiation of meiosis prenatally results from the absence of inhibitory action on behalf of somatic gonadal tissue. Absence of inhibition is typical for the embryonic ovary; it occurs relatively often in sex chromosome chimeric or mosaic testes (being most probably connected with the participation in such a gonad of XX somatic cells), and it can occasionally be produced in purely XY testicular tissue.

3. The available evidence strongly suggests that germ cells that initiated meiosis in the testicular tissue prenatally can occasionally enter into growth phase, but probably degenerate soon afterwards. The majority of these cells, however, degenerate after completing meiotic prophase. There is no evidence that the behavior of germ cells in this period depends on their genetic sex, though such a possibility cannot be excluded *a priori*.

4. In the mouse, and probably in other mammals as well, germ cells cannot complete gametogenesis characteristic for the opposite sex. In the testicular tissue, XX germ cells do not reach the first meiotic division. It remains unclear at what stage of ontogeny these cells are eliminated.

5. The sex-determining genetic factors are more labile in somatic cells than in the germ cells. While the former can survive and contribute to the gonadal tissue of the opposite phenotypic sex, the latter cannot accommodate in such a tissue, and perish.

6. The available evidence suggests that if sex reversal in a mammal were even finally produced, such an individual would most probably be sterile.

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DISCUSSION

Dr. Jost commented on an important point that Dr. Tarkowski had emphasized: that during sex differentiation of the gonad, the germ cells in females undergo meiosis in the early stages, before birth, and in males at about ten days postpartum. Apparently nobody knows what factor controls this difference. Dr. Tarkowski had mentioned experiments in which very young genital ridges from genetically male embryos had been transplanted into older animals with the result that their differentiation had been slightly disturbed, with a number of germ cells entering into meiotic prophase at the time schedule characteristic for ovaries.

Dr. Tarkowski added that the majority of these cells in the male genital ridges degenerate after completing meiotic prophase. In one case the investigator (Ozdzenski) had observed formation of growing oocytes in tissue obtained by this method. The genetic sex of these genital ridges is known because karyological investigation is carried out at the same time.

"Do you believe, then," asked Dr. Jost, "that there is a condition of this experiment by which something that normally inhibits the beginning of meiosis in these male germ cells is absent or deficient?"

"Yes," answered Dr. Tarkowski. "My conclusion is that potentially these male germ cells are capable of starting meiosis at the same time that female germ cells do, but that in the testis they are prevented from doing so."

Dr. Chapeville asked whether Dr. Tarkowski did not think it would be extremely interesting to try to find out the nature of the substances involved in this process. Surely it would be highly important to know how these cells switch from mitosis to meiosis.

"In the ovary," Dr. Tarkowski said, "which is a simpler model, all germ cells start meiotic prophase at approximately the same time. The picture in the male is much more complicated. It is not known whether there is a substance that induces cells to enter into meiosis. This theory is one of two alternatives. The other one, which I lean toward, is that these germ cells have a physiological clock, and at a certain stage of life history they have to enter into meiosis, unless they are inhibited, as they are in the testis."

RNA-DNA: SOME ASPECTS OF DNA SYNTHESIS IN RELATION TO DIFFERENTIATION

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Fore some years, accruing evidence has sugested that DNA synthesis and/or mitosis may be required for progression from one level of differentiation to another. If this is true, it would mean that, at least in some cases, a given cell cannot itself pass through the developmental hierarchy, but that its progeny can so progress if birth of the daughter cells occurs in the appropriate environment. I would like first to consider the four major phases of the cell cycle for future reference.

I shall speak of G-1 cells, or cells in the G-1 phase of the cell cycle, sometimes referred to as resting cells. Apparently during this phase of the cell cycle the decision is made as to whether or not the cell will enter the so-called S or DNA replication phase of the cell cycle. Once the DNA has been replicated, the cell passes through the G-2 phase and then through mitosis. It is during this latter phase that the daughter cells are formed. They then enter G-1, and so forth.

Some cells may remain for protracted periods in the G-1 or resting phase of the cell cycle. However, once they enter into the S phase of the cycle, the birth of daughter cells is usually imminent. Some cells may remain for relatively long times in G-2, but for the most part once cells go through DNA replication, mitosis ensues and daughter cells are formed.

Little is known about what happens during the G-1 phase that determines whether and when a cell is going to enter S or the DNA replication cycle. It is apparent from this cycle that DNA replication is a discontinuous process; it occurs only during this specific phase of the cell cycle.

The conclusion that there may be a coupling between cell proliferation and de-repression of parts of the genome is really based, so far as I know, primarily on circumstantial evidence. I think that more direct evidence will have to be obtained before we can fully accept this interesting possibility. In the context of the emphasis here on environmental influences on genetic expression, I find a discussion of such apparent coupling quite proper.

After we have considered a few examples in which circumstantial evidence suggesting such coupling has been accumulated, perhaps we can speculate on the possible biological significance of this coupling, if it exists, and on the reasons why certain types of de-repression or differentiation seem to require the synthesis of DNA.

The first example I would like to present is based upon work done by Todaro and Green, in which they were studying the transformation of mouse cell line 3-T-3 by the oncogenetic virus SV 40. One of the manifestations of this transformation is that the 3-T-3 cells become resistant to contact inhibition of cell division. The authors report that cell replication is necessary in two connections here. In the first place, in order for the transformation to become fixed, one cell generation in the presence of the virus is required. And for maximal phenotypic expression with respect to resistance to contact inhibition, several generations are required. It is conceivable that the viral DNA exerts this effect by becoming incorporated into the host-cell genome. Unfortunately, there apparently is no information as to whether or not that actually occurs. Nevertheless, it is this kind of evidence that suggests that cell proliferation is required or is coupled to the transformation we are discussing in this context, namely, loss of sensitivity to contact inhibition.

The second example I would like to discuss briefly relates to some work done recently by Griffin and Ber on the effect of hydrocortisone Hela S-3 cells. It turns out that hydrocortisone, or compound F, apparently induces, within these Hela cells, the formation of a new kind of alkaline phosphatase. The authors were able to synchronize the cells in culture and, working with such synchronized cells, report that the initiation of formation of this new alkaline phosphatase within the host cell occurs exclusively during the S phase of the cell cycle. Actually, they did not do an experiment in which they added inhibitors of DNA synthesis, although presumably, if this theory is correct in this instance, the presence of DNA synthesis inhibitors would prevent the initiation of the appearance of this new enzyme. Here we have a case in which there is circumstantial evidence suggesting that there is a coupling between genetic expression—in this case, the formation of a new enzyme—and DNA synthesis.

A third example relates to work done by Eisenberg and Yamata. They have been interested in studying the transformation of iris cells of the newt into lens cells after the lens has been removed. Their observations are that, several days after removal of the lens, the pigmented iris cells begin to make DNA. Prior to removal of the lens in an intact organism, iris cells apparently rarely, if ever, make DNA. After removal of the lens, they subsequently lose their pigment. It was apparent that all of the depigmented iris cells made tritiated DNA in the presence of tritiated thymidine. That is one point.

The second observation was that some of the depigmented cells that had become labeled with thymidine began to make detectable amounts of gamma crystalline. This is a protein that is characteristic of the lens and can be considered, in this context, to be a marker of differentiation in terms of formation of lens cells. The authors pointed out that not all of the depigmented cells went on the form lens cells or gamma crystalline. Their conclusion is that DNA synthesis is necessary, but not sufficient for this type of transformation or differentiation. Again, as in the previous case, no DNA synthesis inhibitors were employed in these studies, so the conclusion that there is the type of coupling we have been referring to is quite circumstantial. It may simply be that DNA synthesis precedes differentiation, but is not absolutely required for it. Again we have a circumstantial situation that seems to point to a coupling between proliferation and differentiation.

Another example that might be considered in this context relates to work that was done by Dr. Wessels and his collaborators. Dr. Wessels has been interested for quite a long time in the differentiation of the acinar cells of the pancreas *in vitro*.

As background material for some of these experiments, I want to emphasize some of the work that was done in this context using fluorodeoxyuridine (FUDR) as an inhibtor of DNA synthesis. Fluorodeoxyuridine inhibits DNA synthesis apparently by blocking one step in the formation of TTP (thymidine triphosphate), which is one of the necessary building blocks of DNA. In the absence of TTP, no DNA can be synthesized. Fluorodeoxyuridine blocks the conversion of d-UMP to TMP. It blocks, in other words, the enzyme that is called thymidylate synthetase. This reaction involves a methylation of d-UMP to give TMP. The TMP is then converted into TTP by introduction of two more phosphate groups.

One other relevant point in methodology is the fact that the simultaneous introduction of thymidine (TdR) and FUDR will short-circuit the inhibitor effect of FUDR on DNA synthesis. The TdR exerts this short-circuiting effect by virtue of the fact that it can be phosphorylated to give TMP, which can then be converted to TTP.

The observations on this system that I wish to call to your attention at this time are really three in number. First, when FUDR is added to an *in vitro* culture system containing rudimentary pancreas, critical mitoses are blocked. In these circumstances it is observed that the formation of zymogen granules by cells within the interior of the rudiment does not occur. FUDR, presumably by blocking DNA synthesis, prevents the formation of zymogen granules.

If at the same time that FUDR is added to the culture, medium thymidine is also added, some differentiation in the form of zymogen does occur. Presumably by virtue of the fact that thymidine can short-circuit the blockage in the formation of TTP, it can and does permit the formation of DNA. In those circumstances, some zymogen granules are formed even though FUDR is present. This points up the fact that few, if any, side reactions are affected by FUDR.

Furthermore, if FUDR is added after the critical mitoses have occurred but before zymogen formation has occurred, zymogen formation will eventually take place. In other words, once the critical mitoses have taken place and before zymogen has been formed, before the translational aspects of the differentiated process have occurred, zymogen formation will take place. This means that FUDR apparently does not block translation or protein synthesis.

Apparently, then, the inhibitory effect of FUDR resides in the fact that it inhibits DNA synthesis. Again, this points up the possibility that it is the coupling between DNA synthesis and differentiation that is the important aspect here.

The last example I would like to bring to your attention relates to work that has been done on the mouse mammary gland (Topper). There are several lines of circumstantial evidence suggesting that for a mammary epithelial cell that is a casein nonproducer to be induced to make casein. [a process dependent upon three hormones—insulin (I), hydrocortisone (F), and prolactin (P)] this cell must give rise to daughter cells. Again, the point is, as I said at the beginning, that it appears as though this cell cannot pass from one level of differentiation to another, but that the next level can be attained in terms of the daughter cells if they are born in the appropriate environment.

The lines of evidence suggesting this are at the moment fourfold. One is that if one studies DNA synthesis as a function of time in tissues derived from mice in different physiological stages, one finds that the peak of DNA synthesis, which in this case is dependent upon insulin, occurs at different times, depending upon the physiological state of the donor animal. But regardless of where the peak of DNA synthesis occurs, the corresponding peak of casein synthesis occurs about thirty hours later.

This was the first observation that seemed to suggest that there might be a relationship between DNA synthesis and differentiation.

A second line of evidence that also suggests that there might be this type of coupling relates to the fact that androgens inhibit the effect of these three hormones on the augmentation of casein synthesis. In other words, androgens inhibit differentiation of the mammary epithelial cell. This depression in the augmentation of casein synthesis elicited by androgens is proportional to the extent to which the androgens inhibit DNA synthesis by the mammary epithelial cell.

One might note that it appears as though androgens do not inhibit the rate of DNA synthesis in a cell that is making DNA. What androgens do apparently, on the contrary, is to reduce the number of cells that enter into the S phase[1] of the cell cycle. In other words, when we observe a 50 percent inhibition in the rate of the incorporation of tritiated thymidine into DNA, that is a consequence, not of the fact that androgens are depressing the rate of DNA synthesis within any given cell, but rather of the fact that androgens reduce the number of cells that enter the S phase.

A third line of evidence that suggests the existence of the kind of coupling we have been talking about relates to the following observation: that one of the three hormone inducers—hydrocortisone—seems to be able to do its job in this context only during the proliferative phases of the cell cycle. That is in contrast to the situation with respect to prolactin. Prolactin can do its job, whatever that may be, post mitotically. It has not yet been determined whether F does its job during the S phase or during the M phase.[2] It appears as though one of the reasons why proliferation is necessary is that one of the known inducers in this system seems capable of acting only during the proliferative phase of the cell cycle.

A fourth line of evidence that is consistent with the possibility that such a coupling exists in this system relates to the studies that were done with colchicine. If one starts with explants derived from pregnant animals, one finds there is at that time a definite, detectable level of casein synthesis. If one cultures those explants with insulin and hydrocortisone, but in the absence of prolactin, one finds that there is a very reproducible fall in the capacity of cells in those explants to make casein; after three days casein synthesis is no longer detectable. Colchicine has no effect on the rate of decline of casein synthesis in this medium. A second pertinent item is that after cells have reached this point, the addition of prolactin causes a re-emergence of casein synthesis. The cells are post mitotic at this point. All DNA synthesis and all mitosis has stopped. So prolactin is, in fact, working post mitotically. If, in addition to adding prolactin at this point, one adds colchicine, there is no inhibition exerted in terms of casein synthesis. It re-emerges just as it does in the absence of colchicine.

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[1] DNA synthesis phase

[2] Mitosis phase

On the other hand, if one adds colchicine pre-mitotically, together with I, F, and P, no augmentation of the rate of casein synthesis ensues. So on evidence of that sort we feel that there is a circumstantial case for the possible existence of coupling between proliferation and differentiation in the mammary gland.

One might ask why proliferation is necessary. I do not really know, and I do not think anybody really knows at this point. There are a number of possibilities that may be considered. It may be that the inducer, in this case particularly hydrocortisone, can exert its effect only on single-stranded DNA. And presumably single-stranded DNA is available in these cells only during the S phase of the cell cycle. Or it may be that the inducer actually works by virtue of gene amplification, which presumably would take place only during the DNA replicative phase of the cell cycle.

Are there any biological implications to such a coupling? The fact is that the factors that determine when or if a cell is going to leave the G-1 and enter the S phase are really unknown. There is some preliminary work going on, but there are no hard facts on that question at the moment. If the kind of coupling discussed really exists, then a cell probably would be more resistant to change or less likely to become de-repressed after exposure to certain environmental factors when it is maintained in G-1 than if it is triggered to go over into the S phase of the cell cycle. In many instances, apparently, nondividing cells are more phenotypically stable than are dividing cells. So it may be that the coupling we have been discussing has a bearing on the sensitivity of cells in terms of genetic expression in response to environmental factors.

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DISCUSSION

"With regard to the effect of insulin in triggering cell replication in the mammary gland, which makes the cell susceptible to hydrocortisone," asked Dr. Feldman, "am I mistaken in citing experiments in which growth hormone has been substituted for insulin and found to elicit replication under these conditions? The cells were not susceptible to the further induction by hydrocortisone and prolactin, which indicates that it is not just a matter of replication per se, but a more specific interaction between the cells."

"It may be that growth hormone will set off replication," Dr. Topper replied. "As Yamata said, DNA synthesis seems to be necessary, but not sufficient. We have some evidence suggesting that insulin has at least three actions in this system. In addition to initiating DNA synthesis, it seems to exert some effect during the DNA synthetic phase itself that apparently is required for subsequent differentiation. It may be that in the absence of insulin this second effect is not exerted, and so differentiation does not occur."

Dr. Topper described an experiment that one can do in this connection. It has been found that mammary gland explants from immature, nonpregnant animals will make DNA just as well in the absence of insulin as in its presence. If, however, the explants are permitted to make DNA in the presence of hydrocortisone and prolactin, but in the absence of insulin, they will not differentiate. Differentiation will occur only if the insulin is there, although quantitatively the insulin has no effect on the extent of DNA synthesis.

Dr. Jost asked for confirmation of his impression that Dr. Topper had said that, in the mammary gland, the hormone acts during the S phase and during replication so that it interferes with replication and causes the change of something into new DNA. "I did not mean to imply that insulin necessarily modifies the genome during the replication of the genome," Dr. Topper replied. "We have no information about that."

Remarking that some of the data presented by Dr. Topper and some in the current literature seem to indicate that the effects are at the DNA, either the single-stranded or the DNA itself, Dr. Kretchmer said that there is probably no question that there are many cytoplasmic changes during the proliferative phase of the cell. It is possible that what goes on in the cytoplasm—and these hormones may in fact be acting at the cytoplasmic level—would produce substances that would act rather as derepressors than to open a genome.

Dr. Topper emphatically agreed. "I am sorry if I implied that something is happening to the DNA itself," he said. "It is now well known that histones are made at the same time that the DNA is made. It may be that some change comes about at the time the histones are being made, a change that results in these effects. As you pointed out, other things are going on during the S phase."

"We know that if you just give insulin followed by prolactin," Dr. Feldman said, "you will not get induction as in the formation of metabolism; you must have the hydrocortisone. As Dr. Jost pointed out, hydrocortisone does some interesting things. It introduces alkaline transaminase. Could you comment on what, in fact, the hydrocortisone does?"

Dr. Topper said that he did not know. He and his colleagues are now examining micrographs of explants that have been incubated in various hormone combinations; they may get some leads in terms of ultrastructures. In the liver system, in which hydrocortisone induces a number of enzymes, its action seems to be independent of proliferation. Its effects occur quickly. There is little proliferation in that liver system. He therefore had not meant to imply that in all systems hydrocortisone requires proliferation for its action.

GENE EXPRESSION IN RELATION TO THE ACTION OF MAMMALIAN SEX HORMONES

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Hormones secreted by the ovary and testis play a paramount role in regulation of the size and functions of secondary sexual organs and of the complex modes of sexual behavior in mammals. It is customary to classify the sex hormones into three main categories: androgens, estrogens, and gestagens. These are operational definitions pure and simple. They are related to activities demonstrable in various bioassay systems, among which considerable overlaps may occur, such as the uterotrophic actions of both 17β-estradiol and testosterone (the principal estrogen and androgen secreted by the ovary and testis respectively in many mammalian species). Designation of androgens as "male sex hormones" and of estrogens and gestagens as "female sex hormones" is infelicitous for a number of reasons. In the first place, all these types of sex hormones can be produced by both the ovary and testis, by the placenta of pregnancy, and by the adrenal cortex of both sexes. Again, gestagenic substances (containing 21 carbon atoms) are biosynthetic precursors of both androgens (19 carbons) and estrogens (18 carbons) in all the aforementioned steroid hormone factories. And responses to sex hormones are relatively independent of the sex genotype of the host—witness the dramatic growth of the male breast induced by estrogens and of the clitoris following administration of testosterone.

The recognition by the middle 1930's that all classes of natural vertebrate sex hormones are steroidal in nature represents one of the most dazzling advances in the history of reproductive physiology. Throughout the evolution of the vertebrates, there seems to have occurred little change in the chemical structure of the major circulating androgens, estrogens, and gestagens that originate from the gonads. The pioneer studies of Sir Charles Dodds revealed that estrogens of high potency (like diethylstilbestrol) that do not contain the steroid skeleton can be readily synthesized in the laboratory, and it is now known that nonsteroidal estrogens are formed in certain higher plants. On the contrary, countless attempts to prepare synthetic substances that lack the steroid ring system yet possess gestagenic or androgenic activities have failed to materialize more than a few very feebly active compounds.

Experiments aimed at elucidating the molecular basis of sex hormone action were not undertaken on a widespread and systematic scale until after the Second World War. Such researchers have naturally been conditioned by the state of understanding of metabolic process patterns in mammalian tissues and by the biochemical fashions of the times. Twenty years ago, two of the central preoccupations of workers in dynamic biochemistry were: (a) group transfer reactions involved in the synthesis and breakdown of small molecules in cells and the production of biologically useful energy in the form of ATP and related nucleotides; (b) characterization of the specific attempts to see if sex hormones might exert their biological actions by influencing or participating in specific enzymic reactions in susceptible cells. At least one example of sex hormones serving of much potential regulatory significance has been authenticated: estradiol-17β and some other phenolic steroidal estrogens can act as hydrogen carriers for so-called pyridine nucleotide transhydrogenase reactions, as catalyzed by a highly purified enzyme obtained from human placenta (Jarabak, Adams, Williams-Ashman, and Talalay, 1962). Although there is little convincing evidence that such hormone-dependent transhydrogenase reactions play a central role in estrogen action (Williams-Ashman, 1965), they continue to excite experimental interest (Karavolas and Engel, 1966). Again, the direct addition of some estrogens, gestagens, and androgens can inhibit or activate a number of enzyme systems studied in the test tube. In some instances, such as the alterations in both the activity and specificity of glutamic dehydrogenase induced by diethylstilbestrol, which was discovered by Gordon Tomkins and his co-workers (see Tomkins and Maxwell, 1963), these *in vitro* effects of sex hormones may be due to their acting as "allosteric modifiers." That is to say, the hormones combine not with the "active sites" of the enzyme protein (which are responsible for binding of substrate molecules), but rather with other, "allosteric sites." When hormones or other effectors interact with these allosteric sites, a reversible alteration in the conformation of the enzyme protein "allosteric transition" occurs, which modifies the ability of the active sites to combine with substrates and hence leads to alterations in the activity and/or specificity of the enzyme. However, all of the allosteric effects of sex hormones that have been uncovered to date appear at best to be of minor physiological significance (Williams-Ashman, 1965). Such studies may turn out, nevertheless, to be useful models for the combination of sex hormones with the "true" receptors involved in the biological actions of these chemical messengers.

Yet the levels of a large number of enzymes in tissues of the male and female genital tracts are known to be influenced by sex hormones *in vivo*. In most cases, such sex hormone-induced changes in tissue enzyme patterns seem to reflect alterations in the rate of synthesis and/or degradation of the enzyme proteins. The explosive developments in molecular biology that have occurred since Watson and Crick in 1953 announced their revolutionary proposal for the structure of DNA (i.e., the genetic material), and since the pioneer experiments of Nirenberg in 1961 opened the door to cracking of the genetic code, have led to the realization that the prime features of genetic expression in living things can be summarized by the simple formula: DNA→RNA→Protein. Three principal types of ribonucleic acids are involved in protein biosynthesis: (a) ribosomal RNA (rRNA) which plays a central structural role in the function of ribonucleoprotein particles (ribosomes) in the cytoplasm, these bodies being the workhorses of protein biosynthesis; (b) messenger RNAs (mRNA), which contain the information (enclosed as linear arrays of nucleotide bases) for the specific amino acid sequences of proteins; and (c) transfer RNAs (tRNA) to which free amino acids initially become attached, and which contain regions (anticodon triplets) that can recognize specific nucleotide triplet codewords in mRNA molecules, and thereby serve to insert particular amino acids in their proper positions in the growing polypeptide chains during protein synthesis. It was quickly appreciated that hormones or other agents that control the production of specific proteins could do so by virtue of affecting: (1) the copying from specific regions of the DNA genome of one or more specific RNA molecules (which might be tRNA or rRNA as well as mRNA species), i.e., "transcriptional processes;" or (2) the transport of these RNA molecules from their sites of synthesis in the nucleus to the loci of the protein synthesizing machinery in the cytoplasm; or (3) "translational" events, i.e., one or more of the complex enzymic reactions involved in the reading of genetic messages by cytoplasmic polyribosomes. These possibilities are obviously not mutually exclusive of one another.

Some early attempts to understand hormonal control of genetic expression were dominated by the notions, advanced by Jacob and Monod, of specific "regulator genes" directing the synthesis of "repressor" molecules, which in turn controlled the transcription of one or more specific mRNAs. Karlson (1963) and others suggested that some growth-promoting hormones may act as "de-repressors." However, the operon-repressor hypothesis of Jacob and Monod originated from experiments on the control of enzyme synthesis in bacteria, organisms that are often forced to adapt quickly to massive changes in their chemical environment. Control mechanisms in the differentiated cells of higher organisms may not only be more complex than in bacteria, but of a fundamentally different nature. As Hotchkiss (1968) has so aptly said ". . . . it is frivolous and irresponsible to propose without evidence that controls of the bacterial type arise autogenously once and for all in embryonic development and did not in any such degree remain responsive to later environments." It is now well established that gene expression in nucleated animal cells involves many features that differ from those in procaryotic microorganisms, such as (a) rRNA molecules being the predominant products of gene transcription in nucleated cells; the rRNAs may turn over more rapidly, and certain mRNAs may be considerably more stable in animal as compared with bacterial cells; (b) only a small proportion of the DNA-like RNA molecules formed in the nucleus of animal cells may ever reach the cytoplasm; (c) the production of ribosomal RNAs involves a complex "processing" in mammalian cell nuclei, whereby very large precursor molecules are chopped up into small units; (d) the transfer of mRNA molecules from nucleus to cytoplasm seems to be brought about by formation of complexes with the smaller of the two ribosomal subunits; and (e) in many mammalian cells, membrane-bound rather than free polyribosomes are the major sites of protein biosynthesis; the lipoprotein membranes of the endoplasmic reticulum may turn over at rates commensurable with those of the ribosomes themselves.

Early changes in the incorporation of labeled precursors into uterine RNA follow treatment of juvenile or oophorectomized animals with estrogens (Hamilton, Widnell, and Tata, 1965; Billing, Barbiroli, and Smellie, 1969). However, it still remains unclear as to whether such swift actions of estrogens can be ascribed solely to an increase in the operation of RNA polymerase reactions in living uterine cells, or may at least in part reflect changes in the pool sizes of precursor ribonucleoside triphosphates. Somewhat later after administration of the estrogens, but detectably within about an hour and maximally at 12 hours, it can be demonstrated that isolated uterine cell nuclei exhibit enhanced RNA polymerase activity, when the latter RNA synthesizing system is measured at low ionic strengths in the presence of Mg ions and under the direction of the endogenous DNA of the nuclear preparations. These are experimental conditions under which material resembling rRNA is the main product. An early increase in the synthesis of what appears to be mainly RNA of the ribosomal type or its precursors seems to underwrite later increases in uterine cytoplasmic polyribosomes, the new polyribosomes having an enhanced ability to incorporate amino acids into proteins, possibly as a result, at least in part, of increases in mRNA production (the latter not being as pronounced as the increase in ribosomal RNA synthesis). Billing, Barbiroli, and Smellie (1969) have concluded that "one of the earliest changes in response to estradiol treatment is a major increase in the synthesis of ribosomal RNA followed later by a similar increase in transfer RNA and by a much smaller increase in synthesis of DNA-like RNA." Whether in these circumstances there is any *differential* change in the production of *specific* messenger RNA molecules is still far from established. But these and other observations on the chronology of changes in uterine RNA and protein synthesis following estradiol treatment fit in well with the classical demonstration by Mueller (1965) that treatment of the animals with the inhibitor of RNA synthesis Actinomycin D, as well as with specific poisons of protein synthesis (such as Puromycin and Cycloheximide) abolish many of the early biochemical effects of estrogens (e.g., enhancement of phospholipide biosynthesis) on the uterus. It is thus apparent that acceleration of genetic transcription occurs before demonstrable increases in gross synthesis during the action of estrogens. Notides and Gorski (1966) have reported, however, that in rat uterus, estrogen can within 30 minutes induce the synthesis of a single protein (separable by starch-gel electrophoresis) well before the well-known overall increase in incorporation of amino acids into proteins.

A rather similar train of events, albeit manifest more sluggishly, can be observed in the ventral prostate or other accessory glands of castrated rats following treatment with androgenic hormones. Orchiectomy of adult animals leads to a rapid involution of these organs with a particularly striking shrinkage of their epithelial cells involving massive collapse of the endoplasmic reticulum, loss of cytoplasmic polyribosomes, and appearance of pyknotic nuclei. Following injection of testosterone into the castrates, increases in the incorporation of labeled precursor into RNA are quickly demonstrable in seminal vesicle, and shortly afterwards, RNA polymerase reactions determined at low ionic strengths are enhanced. Liao, Lin, and Barton (1966) showed that there is a selective effect of testosterone treatment on the synthesis of ribonucleic acids by isolated rat prostate cell nuclei; hormone administration leads mainly to an increase in the production of RNAs with a high guanine and cytosine content that resemble ribosomal RNA molecules. They concluded that the bulk increases in RNA production induced by androgens occur primarily in the nucleolus and reflect transcriptions from only a small region of the total nuclear DNA genome. At later intervals there occur increases in prostatic cytoplasmic polyribosomes with enhanced ability to incorporate amino acids into proteins. There is some circumstantial evidence for increases in mRNA and tRNA synthesis, although not to the same extent as earlier stimulation of ribosomal RNA production; it is still a moot point whether there is any differential alteration in the formation of *specific* messenger RNAs under these conditions. In any event, it appears that in male accessory genital glands after testosterone stimulation, as in the uterus responding to estrogens, transcriptional events occur before enhancement of protein biosynthesis (see Williams-Ashman, 1965; Williams-Ashman and Shimazaki, 1968).

Two important points concerning the foregoing aspects of the action of estrogens and androgens can be raised at this point. First, it must be emphasized that the studies considered deal exclusively with hormone-induced modulations in the biochemistry, size, and functions of secondary sexual tissues, which, in the juvenile or adult gonadectomized animals used as test objects, had long since undergone their initial differentiation in fetal or very early neonatal life. There is abundant evidence that in mammals, where the heterogametic sex appears invariably to be male, the initial differentiation of primitive structures of the female genital tract is essentially anhormonal, whereas androgens secreted by the fetal testis play a mandatory and critical role in the primary differentiation of male accessory reproductive organs. These "organizational" or "morphogenetic" actions of androgens are manifest only during very restricted periods of development and are essentially irreversible. In the latter and other respects, the "organizational" actions of androgens (which also include profound effects of these steroids on development of capabilities for male modes of sexual behavior and of gonadotrophin output by the anterior hypophysis) are rather different from the often reversible "activational" effects of androgens of the type considered above, or comparable "inhibitory" actions of androgens on the hypothalamico-hypophyseal mechanisms responsible for FSH and ICSH output, which occur normally in post-natal life. At present, absolutely nothing is known about the biochemical basis of the "organizational" as opposed to the "activational" or "inhibitory" actions of androgenic steroids. (For a discussion of the biological aspects of the "organizational" actions of androgens see Harris, 1964, and Price and Ortiz, 1965). The question as to whether the molecular events that underlie the "organizational" effects of androgens may, at least to some extent, be recapitulated whenever the "activational" actions of these hormones occur later in life has been raised by Williams-Ashman and Shimazaki (1968).

The second point is that there is no evidence whatsoever that the early increases in nuclear RNA synthesis in uterus or prostate, after their stimulation by estrogens and androgens, necessitate any prior synthesis of DNA. On the contrary, induction of DNA synthesis and subsequent cell divisions are late events in the sex-hormone controlled growth of these organs. Coffey, Shimazaki, and Williams-Ashman (1968) have shown that in rat ventral prostate, DNA synthesis is massively yet transiently increased between the second and fifth day after commencement of daily androgen treatment of castrates. This tremendous increase in DNA synthesis is accompanied by huge elevations in DNA polymerase activity, which fits in with the generalization that in nucleated cells, large quantities of various enzymes involved in the replication of nuclear DNA are elaborated and retained only around the "S" (synthetic) phase of the cell cycle. The timing of the increased DNA synthesis and DNA polymerase activity in the prostate following androgen administration accords with the duration of the hyperplastic phase of prostatic growth in these animals. The factors that determine the restrictions on prostatic growth, which occur despite continual treatment with androgenic hormones, remain shrouded in mystery. In adult orchiectomized animals, some other male accessory sexual organs grow in response to androgens without any cell multiplication and little or no DNA replication; Venable (1966) showed this to be the case, for example, with respect to levator ani muscles of neuter mice. But the important consideration is that the first demonstrable biochemical events during the sex hormone-induced modulations of uterine and prostatic growth and function in gonadectomized adults are manifest before any occurrence of DNA synthesis or cell division. Yet many examples are now known where the appearance of new gene products and the establishment of whole new genetically determined programs of cell function during true differentiation occur only after at least one cycle of cell divisions (e.g., in the differentiation of exocrine pancreas). It is, of course, by no means excluded that in other situations (possibly in regard to the "organizational" actions of androgens on the differentiation of male accessory glands from the fetal urogenital sinus and Wolffian duct), the appearance of new gene products following androgenic stimulation indeed occurs only after the hormones have stimulated the production of daughter cells.

The very swift changes in nuclear RNA production and RNA polymerase activity in uterus and prostate following treatment with appropriate sex hormones cannot be mimicked by direct addition of estrogens or androgens to cell nuclei isolated from these tissues. Furthermore, although no concurrent changes in the levels of enzymes of intermediary metabolism have yet been demonstrable at such short times after injection of the sex hormones, other early changes in the biochemistry of these organs have been described, such as a marked yet transitory fall in the total ATP concentration in rat ventral prostate within an hour after treatment of castrates with testosterone (Coffey, Ichinose, Shimazaki, and Williams-Ashman, 1968). These quick changes in RNA production may therefore be many steps removed, biochemically speaking, from the "primary" sites of action of the hormone.

It is extremely hard to construct theories of hormone action that are not predicated on the assumption that there exist in suceptible cells one or more types of specific "receptor" molecules, which can recognize and combine with the hormone. There are many reasons to believe that such receptors are (a) macromolecules located either on the surface or somewhere within responsive cells; (b) capable of reversible interaction with the hormone via non-covalent forces (Hechter and Halkerston, 1964; Williams-Ashman, 1965). Once combination of the hormones with such receptors takes place, then secondary biochemical amplificatory mechanisms of the type already considered may, in some way or another, be set in motion. This may involve, in some instances, the intermediary production of "second messengers" such as 3′, 5′-cyclic adenylic acid or conceivably polyamines like spermidine, etc., although in the case of androgens and estrogens (unlike some other types of hormones), a few recent claims that cyclic-AMP or polyamines are of central significance in this rest on only very flimsy experimental evidence. In any event, another approach to the molecular basis of sex hormone action has been to search for substances with which the steroids combine in responsive tissues, in the hope that this may uncover the nature of such hypothetical receptor molecules, in addition to nonspecific interactions of the hormones with various intracellular macromolecules, or with enzyme systems that may be responsible for "inactivation" of the hormones or their active concentration (if any) by target cells. Innumerable early attempts to undertake this sort of investigation can be discounted because radioactively labeled sex hormones of only low specific activity were available, so that the hormones had to be administered in unphysiologically high doses. However, recently a number of studies have been published that are more meaningful, insofar as they have employed tritiated estrogens and androgens of very high specific radioactivity that could be given in truly physiological amounts.

The pioneer studies of Elwood Jensen (admirably summarized in Jensen, Suzuki, Numata, Smith, and DeSombre, 1969) have established the following features of estrogen-binding macromolecules (estrophiles) in many estrogen-responsive tissues; similar findings having been obtained in the laboratory of Jack Gorski. In a number of mammalian species, estrogen target organs such as uterus, vagina, anterior pituitary, and hypothalamus contain proteids with a strong and specific avidity for both natural and synthetic estrogens. Tight but reversible associations of these receptors with estrogens (which in the case of estradiol-17β does not involve any chemical transformation of the hormone) appear to be responsible for the striking selective uptake and retention of the estrogens in target cells. The combination of estrogens with these receptors is prevented by estrogen antagonists such as the aromatic substances MER-25, and Upjohn U-11100. Within an hour after injection of physiological doses of the hormone, most of the estradiol-17β taken up by rat uterus resides in the nuclei of both endometrial and myometrial cells. But a fraction of the hormone is bound to a macromolecular component in the cytoplasm. The cytosol of estrogen-dependent cells contains an 8S-receptor proteid that can deaggregate in the presence of 0.4 M KCl into subunits with sedimentation coefficients of about 4S. Estradiol-17β in combination with nuclear protein is extracted by 0.4 M KCl as a 5S-complex, which is distinguishable from the 4S subunits of the cytoplasmic receptor. The cytoplasmic 8S receptor-estradiol complex forms spontaneously, whereas addition of the hormone to isolated nuclei does not bring about formation of the nuclear 5S hormone-receptor complex. Rather, the nuclear 5S complex is only formed in the presence of both estradiol-17β and the cytoplasmic 8S receptor proteid. The latter substance is itself not bound by nuclei unless estradiol-17β or other estrogens are also added. Thus it appears that in uterine cells, estrogens associate spontaneously with a cytoplasmic 8S-proteid "uptake receptor," which then enters the nucleus, by a temperature-dependent process, to give rise to a different 5S-proteid hormone complex that is retained intranuclearly.

It is yet to be determined whether this initial formation of a cytoplasmic 8S-receptor-hormone complex and its subsequent uptake by the cell nuclei to give rise to a 5S nuclear protein-estrogen complex is primarily a device to channel the hormone across the cytoplasm and into the nucleus, or whether the nuclear 5S complex is the "true" receptor for estrogens that may serve to trigger off changes in nuclear RNA synthesis and possibly other early biochemical sequelae of estrogen action. It can also be imagined that the function of the estrogen is simply to aid the transport of the 8S-proteid from the cytoplasm into the nucleus, where it can be transformed (in combination with estradiol-17β) into a 5S-proteid that serves to regulate nuclear biochemical events, the hormone thus acting in a permissive way. At all events, these important new results raise the possibility that the "active forms" of estrogens in their target cells may be this 5S nuclear proteid-hormone adduct. This may have considerable bearing on attempts to influence RNA polymerase reactions *in vitro* by addition of estrogenic substances.

Rather comparable studies have recently been carried out on the fate of labeled androgenic steroids in the prostate gland and seminal vesicle. In many mammals, the principle circulating androgens of testicular origin are testosterone and $\blacktriangle^{4}$-androstenedione. Following injection of these tritiated hormones in physiological doses to castrated rodents, there occurs some degree of selective uptake by the male accessory genital glands, although this is not so dramatic in nature as the selective concentration of labeled estrogens found in the female genital tract. However, in marked contrast to the unchanged nature of estradiol-17β in the uterus, the testosterone or $\blacktriangle^{4}$-androstenedione taken up by the prostate and seminal vesicle undergo rather extensive transformations to a variety of unconjugated steroid products, some of which (such as etiocholanolone) have no biological activity, but at least one of the products, dihydrotestosterone (5a-androstan-17β-ol-3-one), is at least as powerful an androgen as testosterone itself. Bruchovsky and Wilson (1968) and Anderson and Liao (1968) showed independently that a number of androgen-responsive tissues convert testosterone to dihydrotestosterone in high yield. Moreover, these tissues, unlike some other organs like liver, kidney, and brain, selectively retain dihydrotestosterone (but not testosterone itself) in their cell nuclei both *in vivo* and *in vitro*. Male accessory glands of reproduction contain a proteid with a sedimentation co-efficient of about 3S that exhibits a specific and very high avidity for dihydrotestosterone. Combination of this proteid with dihydrotestosterone is prevented by steroidal nonestrogenic androgen antagonists such as cyproterone and cyproterone acetate, these substances having little or no influence on the conversion of testosterone to dihydrotestosterone (Liao, 1969). The latter transformation utilizes TPNH as a hydrogen donor and is catalyzed by a 5a-steroid reductase found in both the cytoplasm and the nucleus of prostate cells.

These exciting investigations from the groups of Wilson and of Liao hint that dihydrotestosterone may be an "active form" of testosterone in some androgen-sensitive tissues. It is of interest in this connection that two recent reports (Mauvais-Jarvis, Bercovici, and Gauthier, 1969; Northcutt, Lothard, and Liddle, 1969) suggest that in the human syndrome of testicular feminization (or "androgen-insensitivity syndrome" as the disease has been re-christened by John Money), there may be considerable impairment in the enzymic conversion of testosterone to dihydrotestosterone. Patients with this condition have a normal XY male genotype and abdominal testes with no great abnormalities in the production or handling of testosterone and no excessive secretion of estrogens; yet they exhibit a completely female phenotype and seem to be quite unresponsive to both endogenous and exogenous androgens. It remains to be proved, however, whether an impaired conversion of tesosterone to dihydrotesterone is the sole or even primary biochemical defect in this syndrome.

The discovery that estrogens in the uterus and dihydrotestosterone in male accessory glands are retained in cell nuclei of these organs in the form of complexes with specific proteid receptor molecules is an important advance, and further developments in this area of research will be awaited with much interest, especially from the standpoint of the possible relation of these receptor proteids to the control of RNA transcription in the nuclei of sex hormone target tissues. Presumably the formation of these receptor macromolecules is dictated by specific genes, the expression of which may be very germane to understanding of critical periods in the responsivity of cells to gonadal hormones.

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DISCUSSION

Dr. Hirschhorn suggested that an experimental system worked on for the last several years might provide answers to some of the questions Dr. Topper and Dr. Williams-Ashman had raised. The system relates to the stimulation of small peripheral blood lymphocytes by various nonspecific stimulants, particularly one called phytohemagglutinin.

The small peripheral blood lymphocyte is a cell that has almost no cytoplasm. It is chiefly nucleus. Within the cytoplasm it does have a few organelles, including ribosomes and a few lysosomes. This organelle contains a variety of enzymes capable of hydrolizing a great number of intracellular substances and is perhaps critical to the argument. It has proteases that degrade DNAse, RNAse, lipases of various kinds, which can completely alter the internal morphology of the cell.

Phytohemagglutinin, if added to a culture of such cells, converts them within a couple of days to very large cells with many cytoplasmic organelles and much cytoplasm. Within the next twenty-four hours, division occurs in a great many of these cells. Normally these cells do not divide unless they are stimulated by a variety of agents.

The sequence of events seems to be something like this: First, the phytohemagglutinin attaches at the membrane. This membrane attachment can be blocked by certain sugars, a point that can be demonstrated quite clearly. Within minutes after this, there is respiration-dependent active pinocytosis of small molecules into the cell. This entry, or perhaps the next step, has something to do with cyclic AMP or with the cyclase, which Dr. Williams-Ashman has just mentioned. But immediately thereafter secondary lysosomes are formed.

This means that there is a merging of lysosomes with the pinocytotic vacuoles that bring in the small molecules. So a different kind of organelle is formed, a merged organelle. In other systems these merged organelles leak some of their enzymes, which can be demonstrated in these cells quite readily. Among these enzymes are certain trypsin-like proteases that destroy or can break up certain proteins.

There is an older study showing that if one takes nuclei, rabbit liver nuclei, for example, and treats them with trypsin, one can increase their so-called template activity in the presence of RNA polymerase. In other words, one can make these nuclei make more RNA by treating them with trypsin. Similarly, in this system, the nuclei following treatment with phytohemagglutinin have an increased ability to make RNA. They have increased template activity in the presence of exogenous RNA polymerase.

It is also known that within the first hour or two one can detect new RNA formation in the cells, the majority of which is ribosomal RNA. Within two or three hours one can detect a significant increase in protein synthesis in these cells.

DNA synthesis does not begin until about thirty-six hours after these events, but almost invariably it follows this sequence of events.

"I think this general system, especially with what is known about the interaction of several of the hormones that have been mentioned with biological membranes and especially with lysosomal membranes," Dr. Hirschhorn concluded, "perhaps might lead to an experimental model for the action of some of these hormones on target tissue."

Dr. Williams-Ashman called this a particularly intriguing possibility. He said: "I think those of us who are interested in tissues of the male and female genital tract, which can wane after removal of circulating hormone and can be made to grow again by exogenous hormones, realize that the importance of lysosomes may be precisely the other way around, that probably an increased formation of lysosomes is very important in the dwindling of these tissues following removal of gonadal hormones. Certainly one can begin to see in the prostate of some species so-called electron dense bodies that appear in the cytoplasm after castration; some people think these are lysosomes."

Dr. Hirschhorn emphasized the fact that this occurs in the cells as well, because at about 48 to 72 hours, approximately, at the time of DNA synthesis, new, massive numbers of electron dense bodies containing acid phosphates—in other words, lysosomes—are formed.

Answering a query from Dr. Hahn about whether the hormone stays in the nucleus for a longer time than others in the tissue, Dr. Williams-Ashman said it tends to be retained selectively in the case of estradiol in rat uterus. For how long the hormone is retained in cell nuclei depends on many factors, including the time it takes for the hormone to get into the nucleus, the route by which the hormone is administered, and in what vehicle the hormone is given. Estradiol may be retained in uterine cell nuclei for many hours after this hormone is given to immature rats in physiological doses.

CHROMOSOMES—GROWTH AND DEVELOPMENT: CHROMOSOMAL INFLUENCES ON SEXUAL DIFFERENTIATION

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The normal human male karyotype consists of 46 chromosomes, 22 pairs of so-called autosomes, which we shall forget for the purpose of this talk, and two sex chromosomes, unpaired in the male—an X and a Y. The female karyotype lacks the Y chromosome and has two X's intsead. Otherwise the complement is identical.

To arrive at such a complement one needs the combination of two cells, each having 23 chromosomes, a sperm and an egg. During the formation of this sperm and egg, of course, the 46 have to split again to make 23 each. In normal gametogenesis, with the sex chromosomes X and Y as examples, there are two divisions resulting in an ovum in the female, containing one X, and sperm in the male, containing either an X or a Y.

Things can go wrong with this process. By means of various mechanisms, abnormal chromosome numbers can be formed. For example in the female, by nondisjunction of two X chromosomes, an ovum containing two X's or one containing no X—a so-called O ovum—can be formed.

In spermatogenesis, it matters whether the nondisjunction occurs at the first or second division. If at the first, if the X and Y stick together, they can make an XY sperm or an O sperm. If at the second division, when the X and Y have already split, an XX sperm and a YY sperm can appear, and an O sperm again.

If a normal ovum is fertilized by any of these four types of abnormal sperm, we get four different types of abnormal sex chromosome constitutions, all of which have been well described. They are XO, XXY, XYY, and XXX.

Similarly, the two types of normal sperm can fertilize the two types of abnormal ova and again produce three of these abnormal genotypes.

The first of these is the XO, with 45 chromosomes, the only case of monosomy of a chromosome known in man, which produces a problem of sexual differentiation known as Turner's syndrome. The illustrative examples are phenotypic females, yet they are sexually infantile. They do not spontaneously develop secondary sex characteristics, though they do have a vagina and a uterus and can be made to menstruate with appropriate cycling hormone therapy. Their breasts can be made to develop with estrogen. They are invariably short—usually less than five feet tall—and many of them also carry several stigmata consisting of congenital malformations such as webbing of the neck, coarctation of the aorta, and a variety of other defects.

Mice have been demonstrated with XO chromosomes. The interesting difference between the human examples of this syndrome and the mouse examples is that the mice, also female, are in fact fertile, or semi-fertile; they have reduced litters, while the human cases are sterile.

The XXY state in human beings presents what is known as Klinefelter's syndrome. These males may look normal, although some may develop female breast tissue. The one uniform finding with them is sterility. They have small testes, lack or diminution of seminiferous tubules and spermatogonia. Similar sterile XXY males have been found in cat, dog, mouse, and pig.

The important lesson learned from the XO and XXY situation is that the Y chromosome in man appears to be responsible for developing a male phenotype. This is a different mechanism for sex differentiation from that known previously for the fruit fly, the species with which most earlier work was done in relating chromosomes to this process.

Another sexual dimorphism related to chromosome constitution was described in 1949. Female nuclei derived from a variety of tissues, such as the buccal mucosa, containing a large, densely staining mass called the sex chromatin at the nuclear rim. These are not found in normal males.

There is one particular chromosome in females that is unusual as to the time at which it replicates its DNA, its genetic material. That is the second X of the female, the one that is not present in the male, the one that is present instead of the Y. It appears to replicate its DNA out of phase with the rest of the chromosome complement and completes its replication much later.

Most XXX individuals are normal females. They have some increased incidence of mental retardation, but most of them appear to be entirely normal. They are fertile. The interesting thing about them is that one finds that a large number of their cells have two sex chromatin bodies. It now becomes clear that the number of sex chromatin bodies that one finds is equivalent to the number of X chromosomes of the individual minus one.

Similarly, if one were to do DNA replication studies utilizing radioactive precursors of DNA, one could show that these XXX individuals have two X chromosomes that replicate out of phase with the rest of the complement. In other words, it would appear that in males and females and all abnormal sex chromosome constitutions, only one X chromosome invariably behaves like the rest of the chromosome complement, while all the other chromosomes apparently replicate out of phase and are represented by sex chromatin bodies.

According to the theory proposed by Mary Lyon a few years ago and fairly well proved in certain aspects, these extra X chromosomes (including one of the two X's in the female) are thought to be genetically inactive in the human being probably after the 16th day or so of embryonic development.

The human XYY chromosome constitution has been in the news recently because these people are supposed to be tall, aggressive, and found in institutions for the criminally insane. That the majority of them are unusually tall can most likely be accepted at this point. Whether or not they appear in institutions for criminals in particularly large numbers remains to be seen. Several recent surveys of newborn babies have demonstrated that this condition may appear as frequently as one in 500 newborn males, which makes them one of the more common chromosome aberrations. If we consider that they will generally wind up in the top ten percent of height, this means that they should appear as one in 50 tall males. In fact, if one looks into the data compiled from prisons, one finds that about one of 35 tall males found in prisons is XYY. It is not yet known whether this figure represents a real increase.

An example of XYY has been found in the mouse. This mouse was sterile, whereas the human XYY is not. But that sterile mouse was not large and not aggressive. So there may be other differences as well.

In the human XYY, meiosis—that is, sperm production—appears to be rather peculiar, for it has been demonstrated that the second Y chromosome gets lost during spermatogenesis. These persons apparently do not have spermatogonia that are XYY. They are XY. This kind of selection against some abnormal chromosome constitutions during gametogenesis appears to be true in the female as well, because in an XXX female one would expect some individuals born to that female to be chromosomally abnormal: some of the ova should contain two X chromosomes. In fact, all of the offspring studied have been normal chromosomally. So it is my guess that the third X also gets lost during oogenesis in these cases by a mechanism that is not at all understood.

There are also problems regarding sex differentiation in the form of morphological abnormalities of the sex chromosomes. For example, there is an abnormal X chromosome called an isochromosome X, which is made up of a duplication of the long arm and a deletion of the short arm.

There are thus a number of abnormal sex chromosome constitutions leading to female sterility and primary amenorrhea. One is the XO, which I have already talked about. There are deletions of the short arm of the X. There are deletions of the long arm of the X, and there are isochromosomes that lack the short arm and have a duplication of the long arm.

It is of interest that all four types of aberrations, in fact, lead to primary amenorrhea, but only the ones where there is a short arm of the X missing show the congenital malformations of Turner's syndrome and are short, whereas those who lack only the long arm of the X are normal in stature and do not have any of the congenital malformations of Turner's syndrome. This indicates that the two portions of the X, very early in embryonic life before the second X gets inactivated (and it is interesting that the abnormal X is always the one that is late replicating or genetically inactive) seem to have quite different functions. One of them, the short arm, is necessary in the female for normal stature and for the avoidance of the stigmata of Turner's syndrome, whereas the long arm apparently is not necessary for this. But both arms of the second X must be present for the normal development of the ovary, because all X anomalies will develop so-called streak gonads and will not develop a normal ovary.

In another morphological abnormality of the X chromosome, there is a ring X that is lacking bits of the short and long arm. The variability in ring X individuals is enormous, some of them even being normal in regard to menstruation, at least for a while, and developing breasts. But most of these have been picked up because they are unusually short, and they also are lacking a portion of the short arm.

There are also morphological abnormalities of the Y. It turns out if the short arm, the tiny bit on top of the Y, is missing, then the person cannot develop into a normal male. The long arm, the biggest part of the Y chromosome, is apparently not necessary for normal male development.

Most hermaphrodites are XX in sex chromosome constitution. The reason for this is not completely understood. Some have proposed a translocation of a portion of the Y to an X, but other genetic reasons may be possible. For example, the Saanen goat carries a gene that leads to the absence of horns. If present in homozygous form in the female, this gene leads to complete sex reversal, except that the hermaphroditic "males" produced are sterile. So there are genes that may interfere with the normal corticalmedullary relationship during development of the fetal gonad.

Another important aspect is the fact that we can have sex reversal in human males. An example is the case of testicular feminization, with XY chromosomes, but with normal female appearance.

There is another type of numerical abnormality, caused by postfertilization events: the production of mosaicism. For example, if we lose a Y chromosome during the first postfertilization division, we will wind up with two cells—XY and XO—producing a type of mosaic. Similarly, we can produce an XYY/XO mosaic by a process known as mitotic nondisjunction during the first or early postfertilization divisions. An example of XY/XO mosaicism may be a child who has a phallus and a vaginal opening and has indeterminate gonads found on laparotomy.

Other examples of XY/XO mosaics include essentially normal male patients with classical Turner's syndrome and a whole variety of intersexes. The reason for this is that the chromosome constitution of the fetal gonad appears to determine locally the direction of sexual differentiation. These cases are also of importance since many of them have intra-abdominal testicular tissue. As is well known, such tissue has a high potential for malignancy.

Another mechanism producing mosaics is that of mitotic nondisjunction. This can produce a variety of double and triple mosaics. Again, using the Y chromosome as an example, such a process can result in an XYY/XY/XO mosaic. One such patient we studied was a phenotypic female, 6 feet 3 inches tall (resulting from XYY), with a gonadal tumor (resulting from XY), and showing primary amenorrhea with lack of secondary sex characteristics (resulting from XO). Such cases indicate that development can be determined by the specific distribution of chromosomally variant cells.

Having looked at these various kinds of chromosomal abnormalities, we may wonder what is the role of the Y chromosome. One strong possibility is that it may contain masculinizing genes. Another possibility is that it may repress feminizing genes on the X chromosome instead of having its own masculinizing genes. A third possibility is that, by some gene product or simply by its presence (and this is not so far-fetched, because in corn merely the presence of a genetically inactive chromosome has an effect) it may cause increased mitotic bursts at specific times in testicular development, and at other times in other tissues, leading to increased height.

This concept of genetically inactive chromosomes having some general effect may be possible because, for example, it has been shown that the greater number of genetically inactive X chromosomes a person has, the lower is the number of ridges in his fingerprints. Therefore, there are quantitative effects attributable simply to the number of X chromosomes a person has.

In summary, sex chromosomes must act at different times during development in different tissues. They act early in development in directing the differentiation of the fetal gonad. They may act at different times, but possibly also exert their effect very early in development, in the matter of the height that the person finally achieves. These various problems of which tissues they act on at what time I think can be resolved primarily by such studies as Dr. Tarkowski mentioned, as well as by careful study of some of the sex chromosome mosaics I have described.

DISCUSSION

If one takes into consideration the relation of the two types of sex chromosome constitution to height in man, Dr. Williams-Ashman asked, would the difference in size that is usual between normal males and normal females hold true across mammalian species?

Dr. Hirschhorn replied that he would prefer to have the comparative anthropologists answer that question. His impression is that in most primates it is true that the male is larger than the female. He was not certain about relative size of lower mammals.

With most rodents, Dr. Levine observed, the male is usually larger than the female. The hamster is the only one he knew of in which the case is reversed.

Dr. Garn thought that there may be possible exceptions to the general rule in mammals. The XY individual, for various unknown reasons, is the bigger one. He is also, apparently in all forms, the delayed one with respect to most discrete developmental phenomena. This is not necessarily true prenatally, where a closure of the palate may occur earlier in the male than in the female.

This is especially true in gonadal development, Dr. Hirschhorn added. The mitotic bursts relating to testicular differentiation occur much earlier than does the reduction of Müllerian structures. Perhaps it is the absence of this initial mitotic burst that later will lead to the retention of Müllerian structure. So the male is earlier in a sense also in terms of some mitotic activity occurring very early in prenatal life.

Thought the male is not much bigger than the female before puberty, Dr. Garn suggested, he is as comparably bigger prepubertally as he is postpubertally if developmental status is taken into account. The ordinary practice of matching males and females by age may be legally and educationally reasonable, but in relation to their developmental progress—ossification, timing, and so forth—annual differences are predicted in a gross way or evidenced when they are matched developmentally rather than sidereally.

As for the question whether the differences in height common in human males and females extend to other species than man, Dr. Tanner called attention to the important sex differences in the development of the skeleton. The human male is retarded as compared with the female. The same thing occurs in the other primates that have been examined from this point of view. It is true also for rats, mice, and dogs. So the differences seem to go quite a long way down among species. With rodents, we find that the first point at which one can demonstrate this developmental timing with the techniques now available is a period somewhat after birth corresponding rather accurately to the time when it can be demonstrated in man and other primates, which is a prenatal time, because men and other primates are born at very different times in the developmental progress.

Questioning the assumption that the increased amount of Y material causes greater height in males, Dr. Tarkowski asked whether anything is known about the height of the few known cases of XX males. Dr. Hirschhorn expressed doubt that investigators accept the existence of XX human males, though there are plenty of XX hermaphrodites who have testicular tissue. Most of them fall into a female range in height.

In response to Dr. Garn's question about the relation of growth hormones in the XYY individuals and time of sexual maturity, Dr. Hirschhorn said that they seem to mature sexually at about the same time as do normal males. But adequate growth hormone studies have not yet been completed, he thought. Studies of testosterone levels in these males after puberty have yielded conflicting findings. Some investigators say that there are elevated androgen levels; others say there are normal androgen levels; and some say there are both. The problem is not yet solved.

Dr. Garn added to Dr. Tanner's observations about skeletal development that the effects of the X and Y combinations are different on different ossification centers. The male is not uniformly retarded compared with the female, or vice versa. In some ossification centers the sex difference is 60 percent; in others it is zero. A whole spectrum of differences in the relative retardation or advancement is introduced by the presence of a Y chromosome. These situations seem to change in cases where there are four X's and the Y. There are complicating factors that must be accounted for. One is that the Y chromosome makes for greater cortical thickness from early life on in all major bones except the skull; the female ends up with a thicker skull. This is one reversal that may be due to the absence of the Y or the presence of two X's, or perhaps to differential development of the brain capsule.

Answering Dr. Martini's query about the situation among pigmies, Dr. Hirschhorn said that the male is taller than the female, that the chromosomes are normal, and the growth hormone apparently also is normal.

"Let me say, incidentally," Dr. Hirschhorn added, "that the fact that the growth hormone is normal does not mean that it is going to act normally. For example, in the case of testicular feminization, which Dr. Price referred to as androgen insensitivity, it now appears clear that the difficulty in these individuals may be an inability to convert testosterone to dihydrotestosterone at the tissue level, and that this is what is responsible for the lack of androgen effect."

Dr. Jensen observed that sexual dimorphism is greater in the primates living in open areas by and large than in those living in the forest. Male and female monkeys and apes living in the forest tend to be more nearly equal in size, whereas the greater differences in size are found in the primates like the baboons that live in the fields. There might be some sort of selection responsible for this, he suggested, on the basis of the need to adapt to more predators in the open areas. But he wondered if there might not be some differences in the karyotypes to account for the different kinds of sexual dimorphism in closely related primates.

Dr. Hirschhorn preferred to accept the evolutionary selective factors rather than to assume karyotypic differences in such a situation.

THE INCIDENCE OF DYSPLASIA OF THE MIDDLE PHALANX OF THE FIFTH FINGER IN NORMAL JAPANESE, IN SOME AMERICAN INDIAN GROUPS, AND IN CAUCASIANS WITH DOWN'S SYNDROME

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A little more than a century ago, J. Langdon H. Down (1866), in a paper entitled "Observations on an Ethnic Classification of Idiots," applied the term "Mongolian" to the type of congenital idiocy that has recently come to be known as "Down's Syndrome" or "Down's Anomaly." (Penrose and Smith [1966] point out that the Russians have for many years applied the name "Down's Disease" to this type of defective.) Down considered the "Mongolian" to be "one of a number of varieties of idiots and imbeciles that can fairly be referred to one of the great divisions of the human family other than the class from which they have sprung." Thus he recognized among such defectives "white negroes of European descent," some of the "Malay variety," as well as others whom he described as "analogues of people who with shortened foreheads, prominent cheeks, deep-set eyes, and slightly apish nose, originally inhabited the American Continent."

Of the varities that Down described in his paper, only the "Mongolian Type" is recognized today as constituting a distinct group. Though it is now generally agreed that the physical resemblance of this type of defective to true Mongoloid people is very superficial, the terms "mongolism" and "mongolian idiot" are still widely used, even by some leading authorities in the field of mental deficiency (Penrose and Smith, 1966).

Thirty years after Down's paper appeared, T. T. Smith (1896) first called attention to the fact that the distal (terminal) phalanx of the fifth finger of many mongoloid defectives inclines towards the midline of the hand (clinodactyly). Smith's contribution is especially important, because his paper includes a reproduction of what is probably the first hand-radiograph that was made of a mongoloid who had this digital defect (Fig. 1).

The illustration clearly shows that this abnormality is not due primarily to a defect of the *distal* phalanx, but rather to a deformity of the *middle* phalanx with which it articulates. In this condition, the distal margin of the *middle* phalanx is oblique, rather than horizontal, slanting towards the wrist and the midline of the hand. Consequently, the distal phalanx that articulates with it becomes deviated in the same direction. The *proximal* margin of the middle phalanx is also oblique, but it slants *distally* towards the finger tips and towards the midline.

Hand-radiographs of young children who have this defect of the middle phalanx of the fifth finger show that the deformity involves the shaft rather than its epiphysis, which assumes a position that conforms to the oblique proximal border of the shaft. As a result, the proximal margin of the epiphysis tends to parallel the corresponding border of its shaft (Fig. 2). Later in development, it becomes more horizontal, due to a somewhat greater growth in its medial than in its lateral half. (Fig. 3 and 4).

Smith believed that the incurved little finger was a distinguishing feature of mongolism, but, as will be shown subsequently in this paper, this is not the case: it is a frequent but by no means a constant part of this distressing syndrome.

In re-examining the hand-radiographs of approximately 1000 normal American-born Japanese children whom we had studied in California some years ago, I was struck by the high incidence among them of an abnormally shaped middle phalanx of the little finger. It seemed of interest to determine whether this digital defect among normal Japanese is the same as that which occurs so frequently among persons with Down's syndrome.

In an attempt to answer this question, we obtained hand-radiographs of 324 patients with Down's syndrome from institutions for the mentally deficient in three different parts of the United States: The Laconia State School, Laconia, New Hampshire; The Pacific State Hospital, Pomona, California; and the Northern and Southern Wisconsin Training Schools. I am deeply indebted to the following persons whose generous cooperation made it possible for us to obtain these radiographs: Joseph Schlessinger, M.D., Deputy Superintendent of the Laconia State School; Stanley W. Wright, M.D., Chief of Research at Pomona State Hospital;

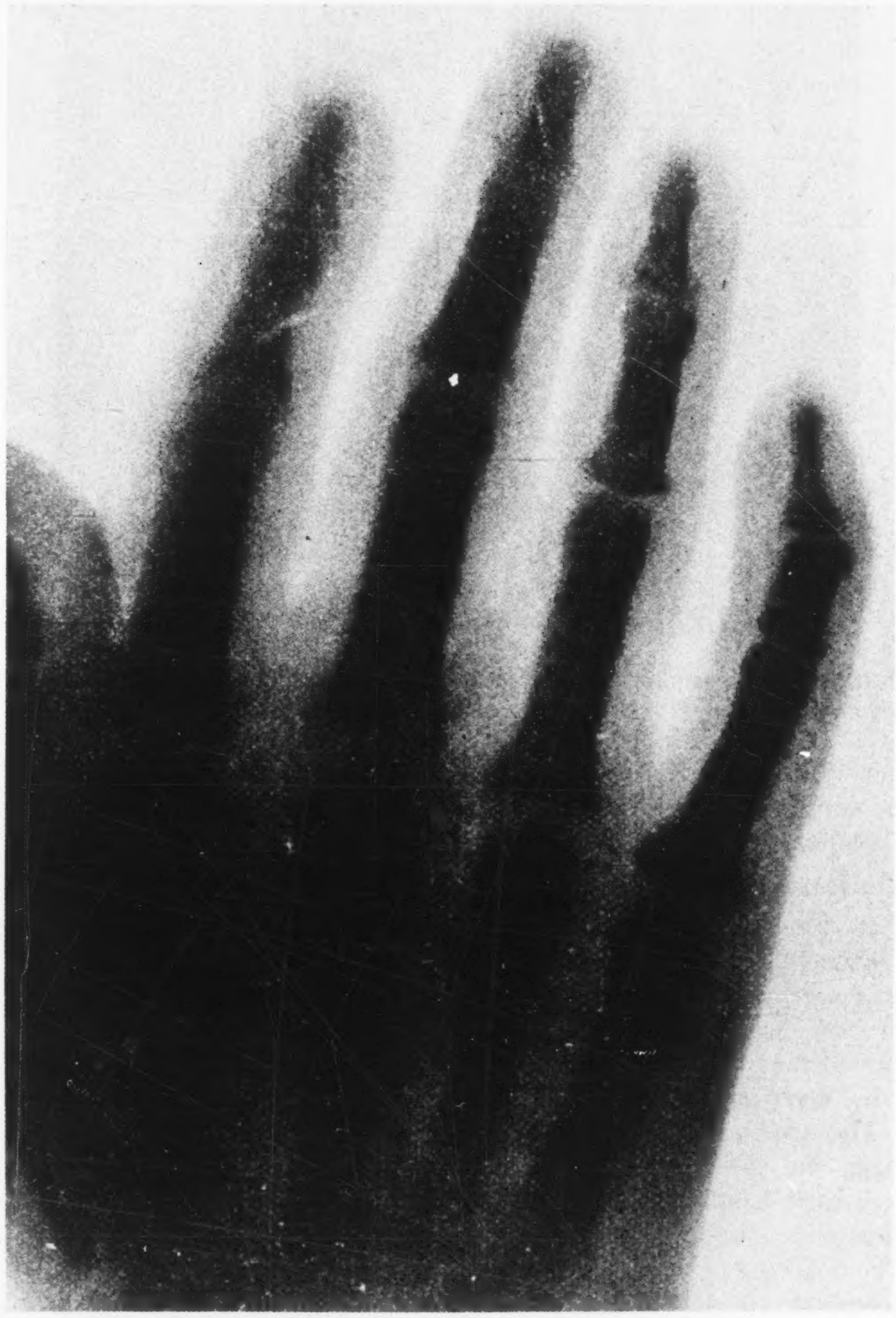

FIGURE 1.—The hand-radiograph of a patient with Down's syndrome reproduced in T. T. Smith's paper of 1901.

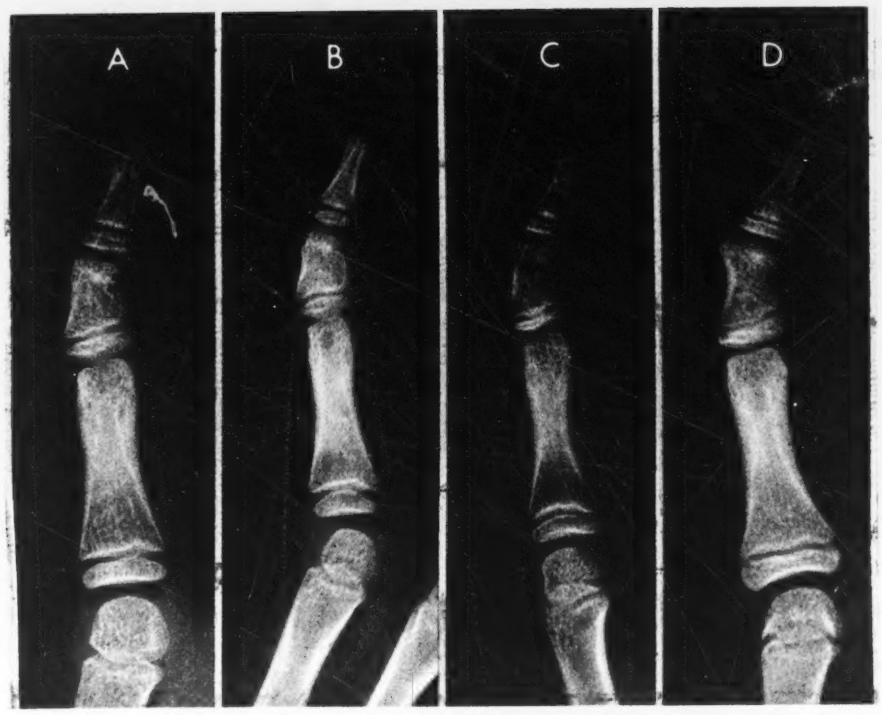

FIGURE 2.–Dysplastic middle phalanges of three normal Japanese (A, B, and C) and one Caucasian boy with Down's syndrome (D).

and G. Laurence Rarick, Ph.D., Professor in the School of Education at the University of Wisconsin.

Findings: (These are summarized in *Table I*)

The Observed Incidence of the Defect Among:

a. *Down's syndrome cases:*

Ninety-three of the 324 mongoloid defectives on whom hand-radiographs were obtained had dysplastic middle phalanges of the fifth finger—an incidence of 28.70%. Of the 183 males in the group 55 (30.05%) showed the defect, while the 141 females, 38 (26.95%) were affected.

b. *American-born Japanese:*

The American-born Japanese group consisted of 990 individuals (516 males and 474 females) who ranged in age from 6 to 19 years at the time their hand-films were made. Two hundred and five (20.70%) of them were found to have dysplastic middle phalanges of the fifth finger. The incidence of this defect among the Japanese showed a marked sexual difference: 69 (13.37%) of the males and 136 (28.69%) of the females were affected.

The observed excess of females among the affected Japanese (but not among the Caucasian mongoloids) suggested the possibility that the defect, though superficially alike in the two groups, might actually be different. It was decided, therefore, to compare the defective phalanges

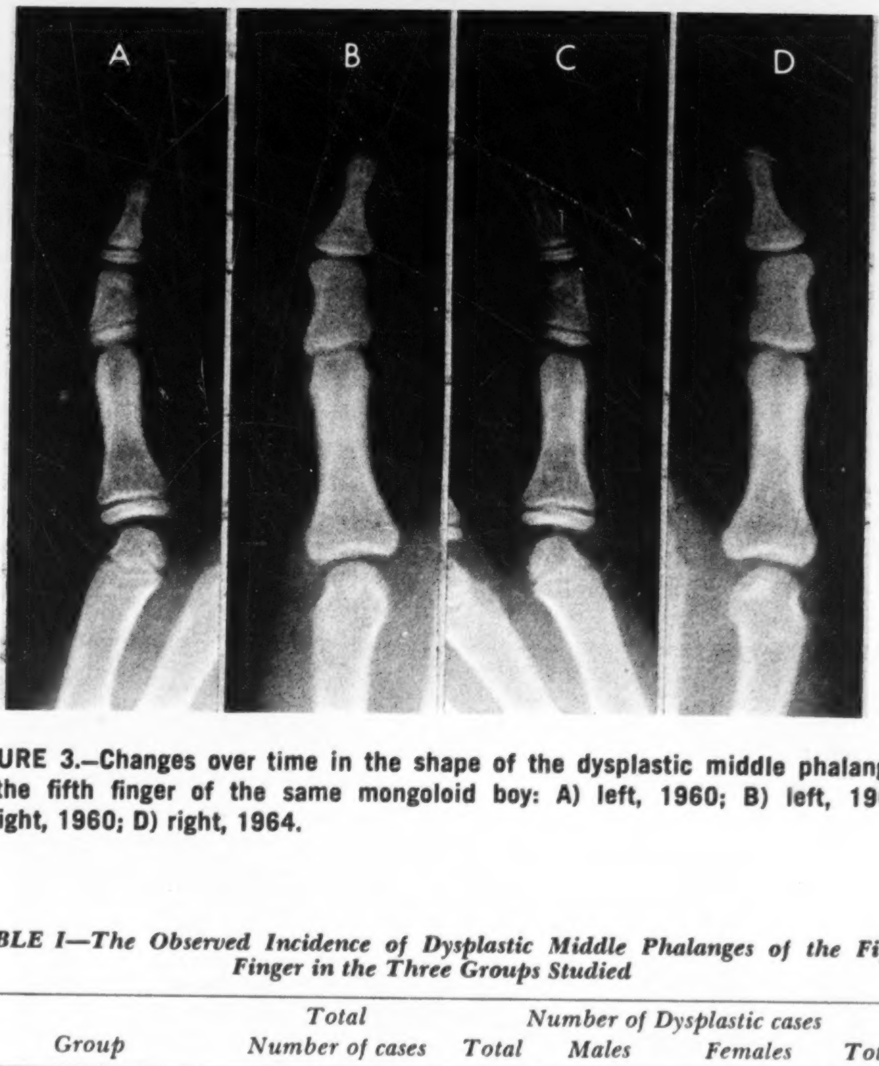

FIGURE 3.—Changes over time in the shape of the dysplastic middle phalanges of the fifth finger of the same mongoloid boy: A) left, 1960; B) left, 1964; C) right, 1960; D) right, 1964.

TABLE I—The Observed Incidence of Dysplastic Middle Phalanges of the Fifth Finger in the Three Groups Studied

Group	Total Number of cases	Number of Dysplastic cases					
		Total	Males		Females		Total
CASES OF DOWN'S SYNDROME				Percent		Percent	Percent
Laconia, N.H.	116 (59 M; 57 F.)	39	18	15.51	21	18.10	33.62
Pomona, Calif.	138 (84 M; 54 F.)	38	26	30.09	12	22.22	27.53
Wisconsin	70 (40 M; 30 F.)	16	11	27.50	5	16.66	22.85
Total	324 (183 M; 141 F.)	93	55	30.05	38	26.95	28.70
AMERICAN-BORN JAPANESE							
JAPANESE	990 (516 M; 474 F.)	205	69	13.37	136	28.69	20.70
AMERICAN INDIANS							
White River Apache ..	250 (113 M; 137 F.)	14	4	3.54	10	7.29	5.60
Pima, Papago, etc.	367 (193 M; 174 F.)	31	13	6.73	18	10.34	8.44
Pueblos	131 (57 M; 74 F.)	13	6	10.52	7	9.05	9.92
Total (Indian) .	748 (363 M; 385 F.)	58	23	6.33	35	9.09	7.75

of the two groups in somewhat greater detail, in order to see if we could detect any consistent morphological differences between them. Some such differences as well as some similarities were noted, and the more striking of them are shown in the accompanying illustrations.

Figure 2-D is a radiograph of the fifth finger of a Caucasian boy with Down's syndrome. The shape of its dysplastic middle phalanx is that observed in the vast majority of mongoloids in our series who had this digital defect. It is also the same type illustrated in Smith's article referred to above and reproduced in Fig. 1. Illustrations A, B, and C of Fig. 2 show the fifth fingers of three *normal* American-born Japanese children. It is quite evident that the same shaped dysplastic middle phalanges of the fifth finger that were found to be characteristic of the affected mongoloids in our series occur also among some quite normal Japanese children and adults.

The degree of dysplasia of the affected middle phalanx of the fifth

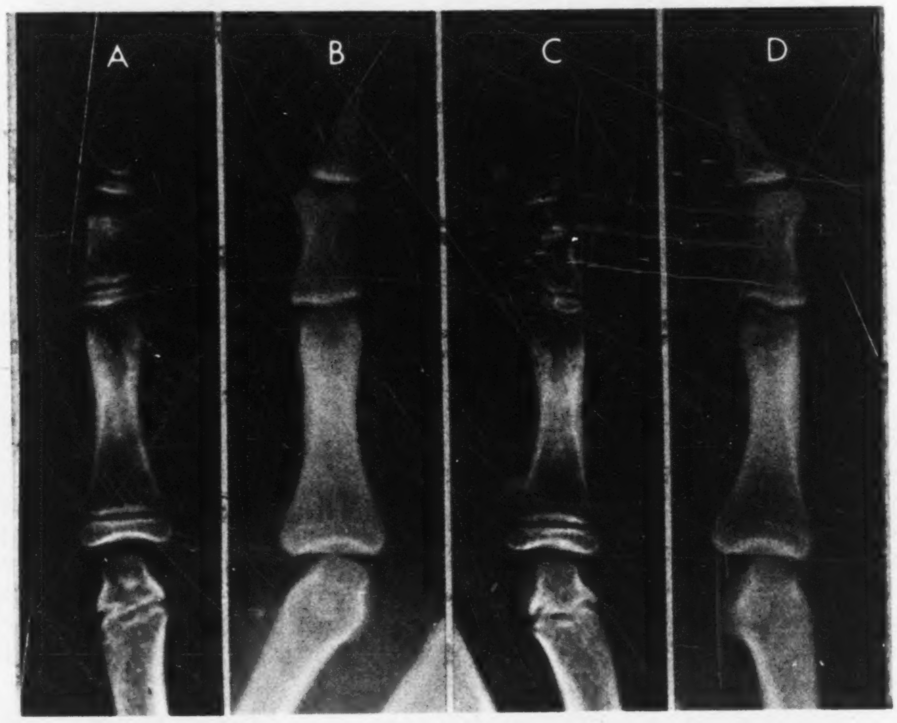

FIGURE 4.–Changes over time in the shape of the dysplastic middle phalanges of the fifth finger in a second mongoloid boy: A) left, 1962; B) left, 1966; C) right, 1962; D) right, 1966.

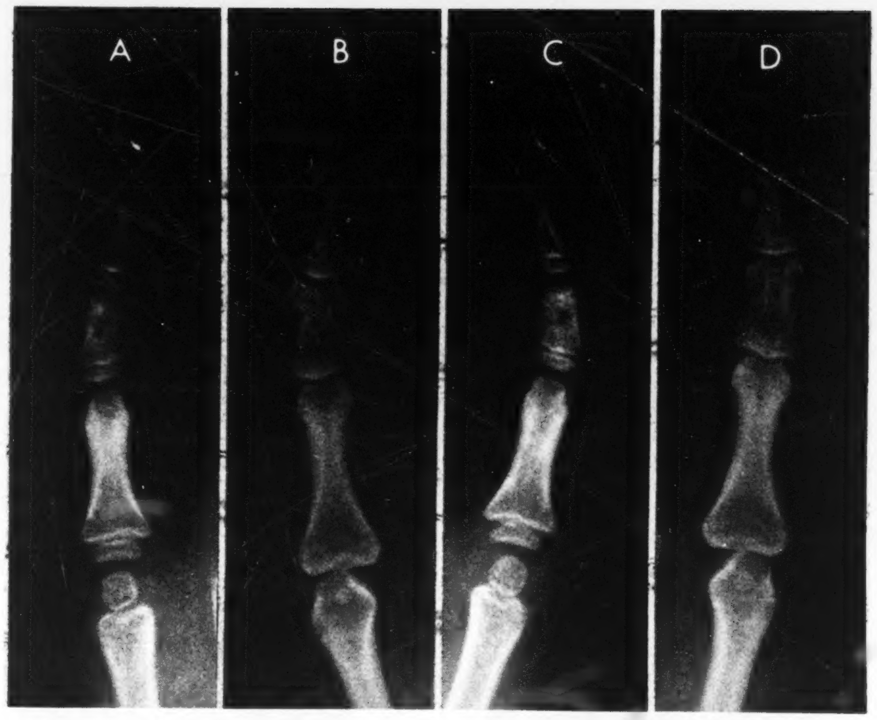

FIGURE 5.–Changes over time in the shape of the dysplastic middle phalanges of the fifth finger of a mongoloid girl: A) left, 1958; B) left, 1966; C) right, 1958; D) right, 1966.

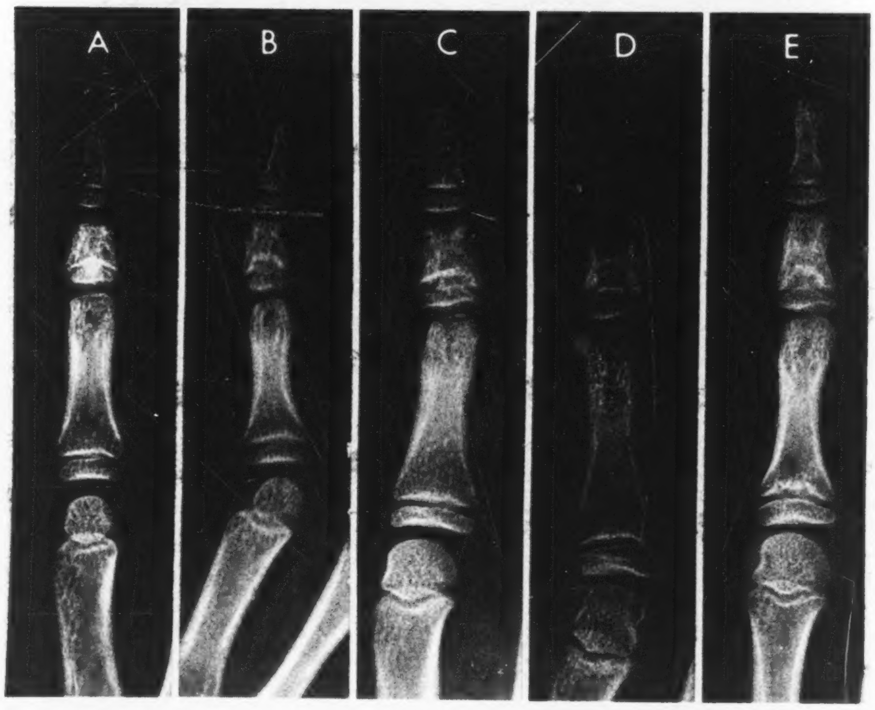

FIGURE 6.–The characteristic shape of dysplastic middle phalanges of the fifth finger in normal Japanese children.

finger is greatest in early childhood and becomes progressively reduced as the child grows older. This is illustrated in Figures 3, 4, and 5, each of which shows two radiographs, made several years apart, of the right and of the left fifth fingers of a mongoloid child.

In Fig. 4, illustrations C and D, show especially well the part played by the epiphysis in correcting, at least partially, the distorted shape of its dysplastic shaft. The same figure shows, too, that the defect can be more marked in one hand than in the other of the same child and, more important, that, after epiphysial fusion is completed, the shape of the middle phalanx is sometimes so nearly normal that it is not readily recognized as being dysplastic. In most cases in our series such a phalanx was found to end up shorter than it should be. Occasionally, however, it is so nearly normal in length that one is hard put to decide whether or not it is (or was) dysplastic.

Since this defect is more easily recognizable in the young than in the adult, it follows that reported data on its incidence can be very misleading, unless one knows the age composition of the group on which they are based.

An examination of the hand-radiographs of the Japanese children in our series who had dysplastic middle phalanges of the fifth finger disclosed that, in the majority of them, the defect took a quite different form

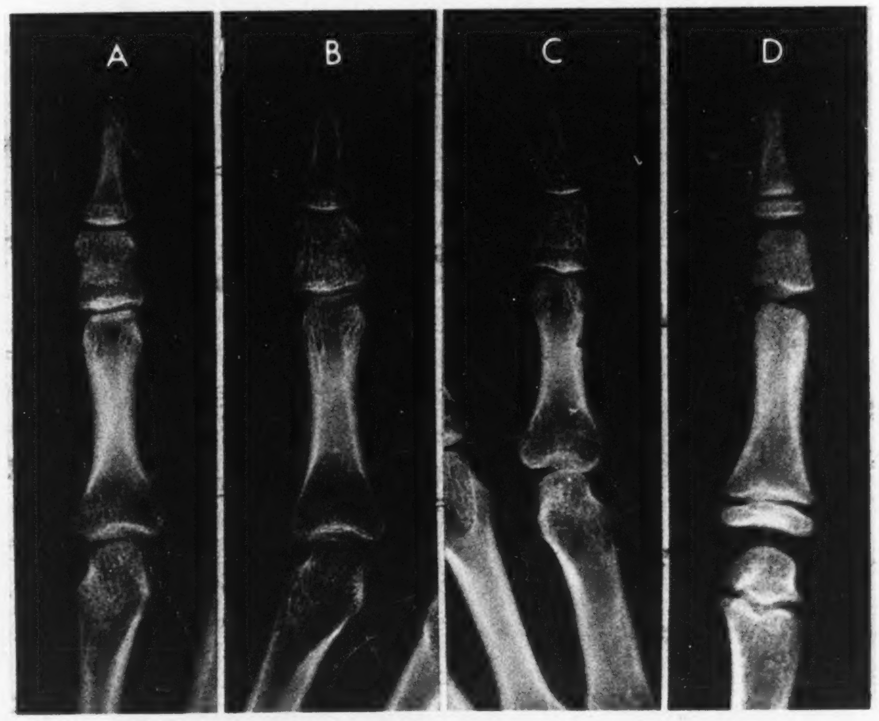

FIGURE 7.—The dysplastic middle phalanges of three normal Japanese adults (A, B, and C) and one Caucasian boy with Down's sydrome.

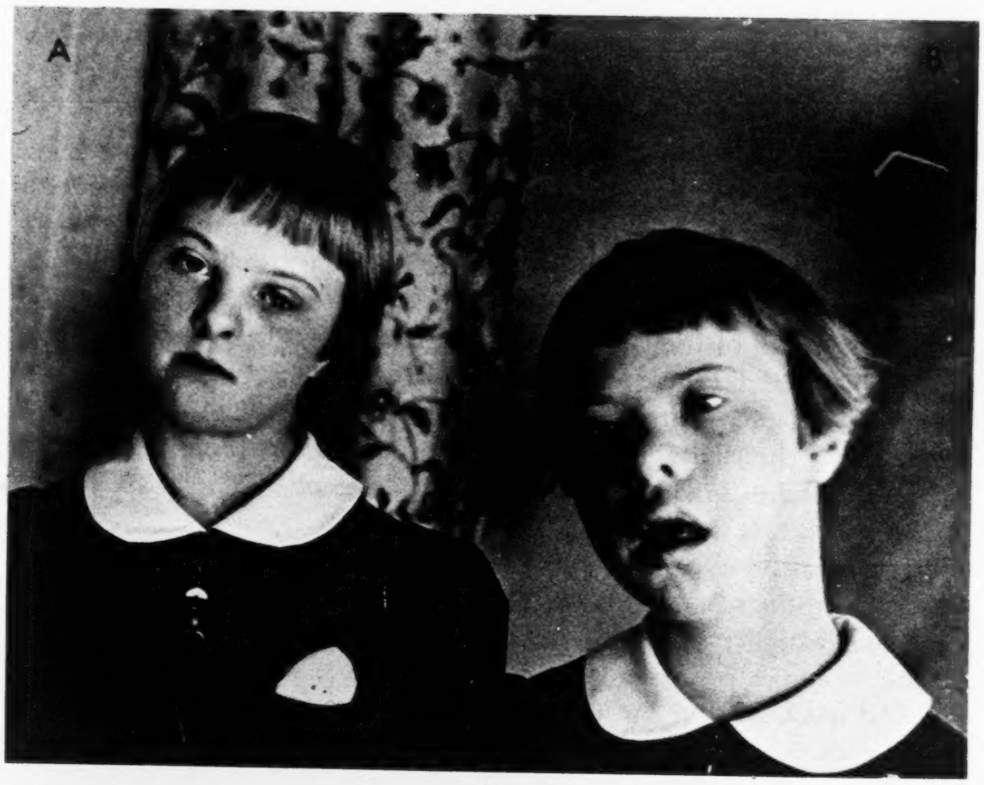

FIGURE 8.—A pair of mongoloid twin girls only one of whom (Twin A) had dysplastic middle phalanges of the fifth finger.

from that which we found to be typical of the similarly affected Down's syndrome patients.

As seen in Fig. 6, which reproduces radiographs of the fifth finger of five mentally normal Japanese children, the dysplastic middle phalanx in that group is characterized by a defective shaft and a mushroom-shaped epiphysis. The shaft defect consists of a concave gap in its base that, in different individuals, varies in the extent to which it extends distally into the body of the shaft. The epiphysis conforms to the shape and size of the gap in the base of the shaft and comes eventually to fill it.

After epiphysial fusion, such dysplastic middle phalanges sometimes end up abnormally short and disproportionately wide, like those of the three young Japanese women whose radiographs are shown in A, B, and C of Figure 7. In other cases, the shortening is not so marked, though the middle phalanges are clearly shorter than normal. If, as occasionally happens the middle phalanx lacks an epiphysis, (as in Fig. 7-D) it ends up strikingly short.

Figure 7 also shows that, in the Japanese, even a severe dysplasia of the middle phalanx of the little finger can be present without any deviation of the end phalanx towards the midline of the hand. Such clinodactyly is, however, almost always present in Down's syndrome patients who have marked digital dysplasia of this type.

As pointed out earlier in this paper, fewer than one-third of the mongoloid defectives in our series had dysplastic middle phalanges of the fifth finger. One can conclude, therefore, that, while the presence of this defect in an infant or young child who, on other grounds, is suspected of being mongoloid, would serve to support that diagnosis, the absence of the defect would not of itself reduce the possibility that the diagnosis is the correct one. Nor did there appear to be any consistent agreement between the severity of the mental retardation or of the mongoloid stigmata and the degree of this digital dysplasia in those Down's syndrome cases in our series who had this defect. The twin girls whose photographs are shown in Fig. 8 illustrate this point.

These children were 10 years and 5 months old when they were photographed and their hand-radiographs made. At that time, Twin "A" was 6 centimeters taller than her sister and, as her facial appearance suggests, she was less retarded mentally than Twin 'B." It was Twin "A," however, who had markedly dysplastic middle phalanges and clinodactyly of both fifth fingers. Her more mongoloid sister was free from these digital defects.

It was also Twin "A" who had a basal (proximal) epiphysis on each of her second metacarpals, in addition to the normal distal one. The fact that Twin "B" had neither of these extra epiphyses nor dysplastic middle phalanges of the fifth finger—features that were so well developed in Twin "A"—was consistent with other morphological differences between them. These considerations led me to conclude that the twins are dizygotic, despite the finding that they were identical in each of six blood factors for which they were tested. Further serological and other studies to determine whether they are identical or fraternal twins are still in progress.

c. *Some American Indian Groups:*

The occurrence of dysplastic middle phalanges of the fifth finger among various American Indian groups has been reported by a number of investigators. Garn and his associates, for example, found this defect in 5% of 238 Quechua Indians from Peru, an incidence that he describes as 50 times as high as that observed among his Ohio reference population. That population consisted of the families of the children who participated in the Fels Research Institute's longitudinal study of human growth and development. Since American Indians are also of Mongolian origin, I was curious to see if this digital dysplasia among them took the same form as it does among the Japanese.

For this comparison, we used the hand-radiographs of 250 White River

Apaches, 367 Pimas and Papagos, and 131 Pueblos, a total of 748. Almost all of the Apaches and Pueblos were adults, but the Pimas and Papagos were children who ranged in age from 10 to 17 years. I am greatly indebted to Dr. J. Roswell Gallagher and the late Dr. Joseph Aronson, who made the hand-radiographs of the Pima and Papago children in connection with a study of adolescence in which we were then collaborating. Mrs. Greulich and I made the radiographs of the White River Apache and the Pueblo adults, as part of another investigation, some years ago.

As shown in Table I, the average incidence of this defect in the Indian groups was 7.75%. This is less than half the frequency of the corresponding defect among the American-born Japanese children in our series, 20.70%. In the Indians, as in the Japanese, however, the defect occurred more frequently among females than among males, except in the Pueblos. It is possible, though, that the Pueblos were not adequately represented in our small sample of them, which consisted of only 57 males and 74 females.

The type of dysplastic middle fifth phalanx that occurred most frequently—indeed, almost to the exclusion of any other kind among the Indians—was found to be the same as that which characterized this dysplasia among the Japanese. Among the Indians, the defect differed from that of the Japanese in that it did not involve quite so much of the shaft of the phalanx and that the concavity in the base of the shaft tended to be more shallow. It was clearly the same type of dysplasia, however, and quite different from that which characterized the defect in subjects with Down's syndrome.

SUMMARY

1. Dysplastic middle phalanges of the fifth finger were found in the hand-radiographs of fewer than one-third (28.70%) of the 324 Down's syndrome cases in this series. This defect, therefore, is certainly not a constant feature of that syndrome. Moreover, it seems to bear no necessarily close relationship to the severity of the associated mental retardation or of the characteristic physical stigmata in this type of defective.

2. When present in Down's syndrome, the dysplastic middle phalanx tends to have a characteristic shape, which is more pronounced and, consequently, more easily recognized in children than in adults. The proximal border of the dysplastic shaft is oblique rather than horizontal, slanting distally towards the finger tips and the midline. Its distal border is also oblique, but it slants towards the wrist and the midline. The distal phalanx that articulates with this border inclines in the same direction.

3. The average incidence of this defect among the Japanese children in our series was 20.70%, as compared with 28.70% in the Down's syndrome cases. There was, however, a marked excess of females among the affected Japanese, 28.69% of the girls but only 13.37% of the boys having dysplastic middle phalanges of the fifth finger. There was no excess of affected females in the Down's syndrome cases. Indeed, the incidence of the defect among the male mongoloids (30.05%) was somewhat higher than among the females (26.95%).

The dysplasia that occurs in the same phalanx of the hands of normal Japanese takes, in most cases, a quite different form from that which it assumes in mongoloid defectives. In the Japanese, the affected phalanx has a concave defect in the base of its shaft and a mushroom-shaped epiphysis that conforms to the shape of the concave defect and finally fills it. In the Down's syndrome subjects, no clear instance of this type of dysplastic phalanx was encountered.

4. The Indian groups included in this study showed a much lower incidence of dysplastic middle phalanges of the fifth finger than did the Japanese (7.75% as compared with 20.70%). The form of the dysplastic phalanges was, however, essentially the same in both groups and quite different from that which occurs so typically in the Down's syndrome.

5. The dysplastic middle phalanx of the fifth finger is another of the superficial resemblances between mongoloid defectives and normal Mongolian peoples. A careful comparison of the form of the dysplastic phalanx and its sexual distribution in the two groups disclose significant differences between them.

6. In Caucasians with Down's syndrome, dysplastic middle phalanges of the fifth finger occur many times more frequently than the incidence of what appears to be the identical defect in normal persons of the same race.

In view of the central theme of this symposium, it is, perhaps, pertinent to speculate whether something in the internal environment of the embryonic or fetal mongoloid produces an increased expressivity of the gene responsible for the defect in some otherwise normal persons of the same race or whether this phalangeal dysplasia in mongoloids has a quite different genetic basis.

The fact that the middle phalanx of the fifth finger is the last of the phalanges to ossify and usually the last in which epiphysial fusion occurs might have the effect of prolonging the period of its vulnerability to any factors capable of distorting its normal growth or differentiation.

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DISCUSSION

Recalling that, before the chromosomal abnormality was known, Dr. Engels at Harvard had pointed out that most of the defects of the child with Down's syndrome were in structures that underwent rapid development in the seventh and eighth week of fetal life and had proposed that nonspecific insults to the fetus at that time would produce this collection of abnormalities, Dr. Barnett asked whether Dr. Greulich had looked for other things that are characteristic of Down's syndrome in the Japanese and Indian children who had shown the defect.

Dr. Greulich replied that so far as he had seen, there are no such stigmata. As he had pointed out, this middle phalanx of the fifth finger is the last of the phalanges to ossify—according to Mall's studies, about day 83. Whether the mechanism that produces this defect becomes operative at somewhat different periods, he did not know. It would seem a reasonable guess, in view of the differences in the end product. One wonders, for instance, why the epiphysis is seldom absent among the Japanese. It comes at the proper time, even though it is larger than it would normally be.

This particular incurved fifth with the defect Dr. Greulich described is common in cases of low brain-weight dwarfism, or so-called intrauterine dwarfism, which would bear out what Dr. Greulich had said about an insult early on in the intrauterine development, Dr. Tanner remarked.

A number of genetically undetermined conditions in which brachymesophalangia 5 occurs in normal individuals are common in the Indians in the highlands of Ecuador and in the Alta Plana of Guatemala as in some groups of Japanese, said Dr. Garn. The defect occurs as a Mendelian dominant that is sex linked. It occurs also in pseudo-hyperparathyroidism to an increased degree, as several people here have observed.

The picture in the chromosomal condition of 21 trisomy not only involves brachymesophalangia 5, but also involves a disproportionate size reduction of all of the middle segments of all the digits, Dr. Garn continued. In Down's syndrome, the relative growth of all of the carpal and metacarpal bones is diminished 15 and 25 percent, depending on the bone in question. So not only is the hand of the adult Down's syndrome person tremendously different in size, but the relative proportions of every other bone differ from the normal by two to seven standard deviations.

Hence the relative growth throughout the hand involving every bone is different. But it is the middle segment of the fifth digit that is so demonstrably different that it is practically diagnostic by itself.

Dr. Hirschhorn said that we have here another bone with a correlation between a single-gene problem and a chromosomal defect: particularly the fourth metacarpal is short in Turner's syndrome and in the XO individual as well as in pseudo-hyperparathyroidism. The fifth can be involved, too. Agreeing with a comment from Dr. Garn that the fourth metacarpal is often very short in the Down's syndrome hand, Dr. Hirschhorn said that this may be a good target organ for anything that interferes with particular phases of development. He thought it a mistake to call such things intrauterine insults. He considered them the result of delays in development. They are quite different from such intrauterine insults as those caused by thalidomide or the German measles virus. One sees the problem in the middle phalanx of the fifth finger and in the fourth metacarpal because for some reason or other they are sensitive target organs in the same way that most chromosomal defects, with the exception of Turner's syndrome, are the result of the brain's having been a target organ, producing mental retardation.

One reason it appears, however, Dr. Garn added, is that those are very apparent bones. He and his colleagues had spent about a year measuring the ratios and sizes of all the bones to each other. They found, for example, that the metacarpals are affected, as Dr. Hirschhorn had noted. The carpals are affected also. But the carpals are ordinarily such funny-looking bones that, except in something like Morquio's syndrome, one does not know how funny they are. In brachymesophalangia 5 and reduction in 3, 4, and 5 with the carpal or metacarpal size, with the ulnar size, there are changes in the carpals, there may be reduction in size of the distal row of carpals on the ulnar side as well, leading to an ulnar deviation. There also may be reduction on the radial side of the proximal row of carpals in some conditions.

What we ordinarily see in looking at the X-ray, Dr. Garn continued, is the bone, or bones, that stand out are most different in our eyes; but really a number of bones are affected at once. When we have ways of measuring them, we see how much has happened.

Dr. Greulich said that one of the characteristics of this syndrome is the fact that descriptions in the text are merely a catalogue of all the defects that have been seen in children with Down's syndrome. But the degree of development of any one of these may vary tremendously. One may be extremely pronounced, and another, which according to the list is just as important, is not there at all. So one would expect to find the same sort of thing that he and his colleagues had observed in the more retarded twin: that her bones were much more nearly "normal" than were those of her less retarded sister.

In answer to Dr. Hirschhorn's inquiry about the basis for calling those two girls fraternal twins, Dr. Greulich said that they had the history of the delivery, which Dr. Hirschhorn considered insufficient evidence. "One would need actual genetic markers," he said. "Just seeing the placenta without detailed histological studies of it is not enough."

Dr. Hirschhorn and Dr. Greulich discussed the possibility of the girls' being monozygotic twins, since, as Dr. Hirschhorn remarked, it would be unusual to have nonidentical twins both of whom were mongoloids.

Another reason why Dr. Greulich felt certain that the twins were not identical was the difference in shape of various bones of their hands. The hand bones were much more dissimilar in these twins than is the case in identical twins. Dr. Hirschhorn observed that children with Down's syndrome may have different degrees of developmental defect. Dr. Greulich noted that in most of the identical twins whose hand-films he had studied, the shape of the individual bones was very similar in the twins and that there was often an amazing similarity in the trabecular pattern of individual bones. This pair of twins did not show such similarities.

Dr. Barnett thought that the principle of intrauterine insult had not been substantiated. But he would defend staunchly the principle of codeterminants in producing abnormalities and the principle that, with the genetically determined disorder, other determinants may be required for it to manifest itself. If he had understood Dr. Hirschhorn's point, it was that this was a developmental phenomenon or arrest rather than an intrauterine insult. Dr. Barnett thought it could be both.

Dr. Garn said that in reviewing cases of Down's syndrome that he had studied in earlier years, he had found that some of them antedating present knowledge of the chromosomal basis for the syndrome had marvelous case histories describing all kinds of things that had happened to the mother while she was pregnant. Since the chromosomal basis of Down's syndrome has become accepted, many of those histories are now reduced by 80 percent. In the days when people followd Engels' work and hypoxia and other things, histories supported particular events as bringing about the syndrome; today one no longer finds the information reported.

Dr. Barnett remarked that, as he saw it, when Engels had made his observations, everyone was looking for something that happened during the seventh and eighth weeks of pregnancy. Now that everyone knows about the chromosomal abnormalities, there may not be enough attention paid to the pregnancy history. Hence everyone assumes that the syndrome is entirely explained on the basis of a single determinant. Dr. Garn agreed.

Dr. Hirschhorn added the suggestion that the presence *in utero* of a child with trisomy 21 may alter the pregnancy history.

DEFICIENCY OF 3β-HYDROXYSTEROID DEHYDROGENASE

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I shall first review a bit about congenital adrenal hyperplasia, and then focus upon the 3β-hydroxysteroid dehydrogenase deficiency.

As an example of the commonest expression of this disorder, we have a female born with a certain ambiguity of the genitalia so that in great measure she resembles a male, though she is female in all senses, including chromosomes. Another example is the case of a four-year-old boy with the disease who was normal at birth, but in early life developed sexual precocity that was not constitutional in that his testes were tiny. Therefore, the androgens were coming from some source other than the testes.

The characteristic mark of the commonest form of the disorder is demonstrated in this way: if one extracts the steroids from the urine of a person with the disease and reduces them with borohydride, then oxidizes with periodate, one finds a tremendous amount of acetaldehyde released. All these compounds lack an oxygen on the 21 carbon of the steroid molecule. This has led to the designation 21-hydroxylase deficiency. We do not know a great deal about these enzymes, but at least we shall refer to them in terms of the position on the molecule that they hydroxylate.

The molecule goes as far as 17 hydroxyprogesterone and is blocked. Then it is excreted in the urine as pregnanetriol, which is one of the indications of the disease.

For many years, the reason for the congenital virilization, which was progressive at birth, was not entirely clear. Some thought it was this molecule itself, which was androgenic, although this is not really so. As it turns out, when this material piles up, although most of it is excreted as a reduced product, much of it is then diverted into the androstenedione→ testosterone pathway. It probably is the testosterone itself stemming from this diversion that explains the virilization.

The other peculiarity about this disease is that, as most of us realize, in 80 percent of the cases, the block is not complete. On careful study it turns out that respectable amounts of the subsequent substances are secreted, but at the expense of very high ACTH levels, the expense of tremendous increase in adrenal protein synthesis, and the expense of a pileup of a great amount of this particular precursor.

This, then, is the commonest form of the disease. But we do not fully understand why 80 percent are what I call compensated clinically because they do break through, and why 20 percent are completely blocked, theoretically in more trouble than those I shall refer to as partial block.

The enzymes hydroxylate at the 17th carbon and then the 21st and finally the 11th. In addition to the elevation of prenanetriol, there is also an elevation of 11-ketotriol, pointing to the fact that 11β-hydroxylation was going on a prior molecule in the absence of 21-hydroxylase. Thus the sequence of enzymatic specificity is not of a very high degree. This has a bearing on the 3β-hydroxysteroid dehydrogenase deficiency.

The adrenal cortex is variously hampered, because 80 percent give evidence that they have some enzymatic activity based on the secretion of various of the steroids, both at the point of block and beyond it. If the defect is severe, there is a great threat to life. There is a compensation with excess intermediary metabolites in alternate paths.

But certain enzymes are present in both adrenal and gonads. This point escaped us for many years because there was no good reason to become involved with 21-hydroxylase in the gonad or, for that matter, with 11-hydroxylase. The absence of these two enzymes was better understood in the beginning simply because neither of these enzymes plays a very important role in steroid genesis in the gonad. In fact, they are hardly present.

But as we come to some of the more recent forms of congenital hyperplasia, it turns out that other enzymes are present in both tissues. From a study of human disease, it would appear very likely that they depend on the same gene because, when they are absent from the adrenal, they are also absent from the gonad. When this is true—when the enzyme is also absent from the gonad—there is no virilization because the same enzyme is essential to the secretion of testosterone itself. Now we run into the clinical exception. Thus we can no longer refer to congenital adrenal hyperplasia as virilizing congenital adrenal hyperplasia. It is not always virilizing.

The first exception that we ran into was a male in all respects, with complete hypospadias, who was said to have the disease because the urinary 17 ketosteroids were elevated. This was not consistent with the hypothesis that large amounts of testosterone were being produced, large enough, indeed, to bring about virilization of the external genitalia of the female.

If this hypothesis were universally applicable to all of the diseases represented as defects in cortisol biosynthesis, this simply should not happen. There should not be a male who did not undergo a complete realization of the formation of the normal external male genitalia. We studied this male very carefully, and there was no evidence that he was anything but a male with complete hypospadias. The case, therefore, required extremely careful study of the urine.

All the compounds in the urine of this subject retain the δ-5-3β-hydroxy configuration of cholesterol itself. Cholesterol is a very primitive substance on the biosynthesis pathway to cortisol biosynthesis. These compounds also are sometimes associated with adrenal tumors. They are the compounds that give the so-called Allen blue reaction in urine if present in large amounts. But we would regard them as "abnormal" steroids that have no business being there in any but relatively small quantity. When they predominate, it seems to point to a definite deficiency.

The particular deficient enzyme that operates here oxidizes the OH at the 3β position.

There is a second enzyme, the so-called transisomerase. I do not know how important it is, because I know that if we oxidize δ-5-3β steroids with chromic acid in the test tube, the double bond switches over without any additional assistance. But at any rate the key enzyme appears to be the dehydrogenase. Without it the primitive ring AB configuration is retained. Without that enzyme it is impossible to get down to the region of the biosynthesis of the sex hormones, including testosterone itself.

The other problem with such an early block is that for some not entirely clear reason it is incompatible with life. Virtually all the cases we know of died in the first year of life despite vigorous treatment with what would be regarded in most circles as adequate replacement therapy with the key substances such as salt-retaining steroids and cortisone. The reasons for this are not clear.

Dr. Gallagher suggested to us that this was because of an inevitable disorder in bile acid metabolism. We studied this very carefully, thinking we might find a defect in the degradation of cholesterol to bile acids. After two years of hard work, I report that there appears to be no defect in bile acid metabolism. Therefore, it would appear that we have other evidence that the enzyme involved in bile acid metabolism is not the same as the one that plays a role in adrenal steroidal genesis.

This enzyme appears to be absent in the subjects with this disease. Then an extraordinary thing happens. The various hydroxylases go on to perform their duties so that one gets a sequence of compounds that resemble the normal ones except that they retain the primitive δ-5-3β configuration. They do not have biological activity required for life maintenance.

Dehydroandrotestosterone is the major C-19 steroid produced but is very weakly androgenic, and not sufficient to bring about full masculine development. The females with this form (we have had only three) are mildly virilized. They have some growth and some fusion of the labia, but the urethra is in the normal position.

We looked at this further and measured the enzyme in some tissues. By direct study of gonads and adrenal tissue from affected subjects, we found it to be absent.

In the normal or ordinary form of adrenal hyperplasia, there is plenty of the enzyme. This is a special case. One can see that the other enzymes are present, but this one is absent in the tissues studied.

Certain tumors in the adrenal cortex are also deficient in this enzyme, which no doubt explains the reason for the high level of the primitive steroid structures in the urine of persons with such tumors. It is a convenient way to diagnose the tumors. Other tumors in patients whose urine does not show such substances have large quantities of enzyme in them.

On clinical grounds, therefore, one has reason to suppose that there is some testicular deficiency as well as adrenal. When one examines the tissues of the testis one finds that the enzyme is missing there as well as in the adrenal cortex.

We know that there are five forms of congenital adrenal hyperplasia. Only two of them are associated with virilization present at birth or progressive later on. I call 3β-hydroxysteroid dehydrogenase barely virilizing, by which I mean that the females are slightly virilized and the males are not sufficiently virilized. They are sort of held off in an in-between stage.

Then there is the 17-hydroxylase deficiency recently described by Dr. Biglieri in San Francisco. The position here is the same as this enzyme—namely, that 17-hydroxylase is necessary for sex hormone biogenesis. Therefore, there would not be virilization. In the desmolase deficiency, which Dr. Prader has described in great detail and which is the earliest of all the blocks, the gonads also appear to be involved. This, too, is incompatible with life, strangely enough. All the cases I know of—there are not many—have died shortly after birth. This presumably is the block right at the point of cholesterol. The adrenal is unable to cleave the side chain.

We have been able to produce an animal model of the 3β-hydroxysteroid dehydrogenase syndrome in rats. This has been done through the administration of a synthetic analogue, 2α-cyano-4,4,17α-trimethyl-17β-hydroxyandrost-5-ene-3-one. It has little or no androgenic activity. When this substance is administered to pregnant rats at certain critical periods, somewhere around day 15, there is hypospadias in the male. This period corresponds to the time at which the enzyme itself is at its maximal level in the fetal adrenal. Furthermore, when we examine the adrenals of mother and newborn rat, we find the enzyme to be absent by use of the diformazan deposition technique. The inhibitor is rather unusual; it is stoichimetric and it appears to be irreversible. The enzyme remains absent from the tissues months after the administration of the analogue. The analogue inhibits not only the mammalian enzyme but also the bacterial. In the female newborn rat there is some slight virilization, including clitoral hypertrophy.

DISCUSSION

Dr. Hirschhorn asked whether anything is known about structure of cell membranes or other membranes in some of these early blocks, in view of the fact that perhaps cholesterol, or something made shortly after cholesterol, makes up some of the basic structure of certain biologic membranes. He was interested in trying to find out something about the cause of death of these people, since Dr. Bongiovanni had only some general causes. He wondered whether there were some ultrastructural problems rather than obvious ones.

"Something of this type must be injected," Dr. Bongiovanni replied, "and we have not looked into it. I agree that there could be several blocks. The block might be in the 20 hydroxylation. You have to hydroxylate cholesterol at 20 and then at 22 and then split it. It could be in any of these three places. I have no idea how this would affect membrane structure."

Dr. Chapeville asked whether the individual contained very small amounts of enzyme or none at all. Had the enzyme been purified? Dr. Bongiovanni replied that it had been only relatively purified. He added that there is a bacterial enzyme as well. To Dr. Chapeville's question whether the diagrams shown were some background, Dr. Bongiovanni answered that they were not intended as background. "It is an upper and lower range." "So there is some enzyme; it is not an all or nothing phenomenon," said Dr. Chapeville. Dr. Bongiovanni thought that in their cases it was practically nothing—at the lower limit of their ability to measure it. "I would want to be cautious," he added, "because there are instances of a disease having been described because it was an obvious, complete disease, and then someone discovered that there were far milder causes of the same disorder."

"Are the cases found in some families?" asked Dr. Chapeville. Dr. Bongiovanni replied that they had had two families with the disorder. A surviving patient of Dr. Migeon's has only a partial block. There was some enzyme, but the patient has many problems similar to those Dr. Bongiovanni had described. He has survived for fourteen years.

"I wonder if there may be any evidence that this is not an enzyme deficiency, but a truly altered enzyme," Dr. Williams-Ashman said. "Some of these hydroxysteroid hydrogenases are very unstable enzymes. With just such a study of its stability, would it be possible to get some idea as to whether this is an alteration in the expression of some particular protein or really an altered protein? Has anybody looked at the stability of this enzyme in the situation where there is only an apparent partial deficiency?" Dr. Chapeville asked whether Dr. Bongiovanni agreed that it is a mutant.

Answering that he did so agree, Dr. Bongiovanni remarked that this particular enzyme is extremely stable. The others have not been looked into very carefully. There is a bacterial enzyme that works exactly the same way, but he and his colleagues know that structurally it is very different. It has been studied at Brandeis University. In a test tube one cannot distinguish the two enzymatically. They work the same way.

Dr. Jost asked a question relating to the last slide shown. When the antagonist is given to pregnant rats, how is it that the mother animals typically use the progesterone? Dr. Bongiovanni said it was a good question, and he did not know the answer. Dr. Martini asked whether progesterone was really essential for the pregnancy of the rat to continue to the end. Dr. Jost replied that it was. Some fetuses may survive without it, but they were unusual cases.

Dr. Bongiovanni added that he and his associates have a very high rate of abortion in these animals—about 50 percent—and a high rate of mortality in the newborn rats. Most of them die within a day or two of birth.

PHYSIOLOGY: HORMONAL INFLUENCES ON THE DEVELOPMENT OF THE HYPOTHALAMUS OF THE RAT

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INTRODUCTION

The hypothalamic mechanisms that control the secretion of pituitary gonadotropins operate in a different fashion in male and female animals. Recent data indicate that the differentiation of these mechanisms into a male or a female pattern does not depend solely on genetic factors; apparently a major role is played by the hormonal milieu existing during particular periods of the fetal or neonatal life.

Gonadotropin Secretion Patterns in Genetically Male and Female Rats

Pituitary gonadotropin stores in adult animals: After puberty, the amounts of luteinizing hormone (LH) and of follicle stimulating hormone (FSH) stored in the anterior pituitary are more elevated in male than in female rats (Lisk, 1968; Kragt and Ganong, 1968 a and b). On the contrary, the concentration of the follicle stimulating hormone releasing factor (FSH-RF) in the hypothalamus is much higher in females than in males (Martini, Fraschini, and Motta, 1968 a and b).

Pituitary gonadotropin stores during sexual maturation: During sexual maturation, the concentrations of LH and of FSH in the pituitary follow different patterns in the two sexes.

In males, LH rises from birth to about thirty days of age; it shows a small drop between day 40 and 50 of life; then it returns to rather high levels and remains elevated for the rest of the life of the animals (Lisk,

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* With M. Motta and F. Piva (Ford Foundation Fellow).

1968). In females, LH rises from birth to puberty; at that time it reaches much higher levels than those found in males of the same age. At time of vaginal opening, there is a critical drop of pituitary LH content; this remains very low from that moment on (Lisk, 1968). It is apparent from these data that, from birth to puberty, the concentration of LH in the female is significantly greater than in the male, and that this relationship is reversed following sexual maturation.

Similar findings have been reported with regard to FSH. In female animals the pituitary concentration of FSH rises from birth to the time of puberty; pituitary FSH content declines thereafter and remains low in adulthood (Corbin and Daniels, 1967; Kragt and Ganong, 1968b). On the contrary, the FSH content of the anterior pituitary glands of male rats increases from 10 days of age to 35 days of age; thereafter it levels off at a level that is 15-20 times the value of adult females (Kragt and Ganong, 1968a).

Response of pituitary gonadotropins to feedback stimulations: Castration is followed by an increase of pituitary FSH stores in rats of both sexes; however, the increase is dramatic only in female animals, probably because of the very low departure levels. Following castration, there is also a significant increase of FSH-RF hypothalamic stores. This is evident in both sexes, but is higher in males. (Martini *et al.*, 1968 a and b).

Estradiol significantly reduces pituitary stores of FSH and hypothalamic stores of FSH-RF in castrated rats of both sexes. Testosterone decreases FSH and FSH-RF contents in castrated males, but is ineffective when administered to castrated females. The opposite has been found after administration of progesterone. This steroid is highly inhibitory on both FSH and FSH-RF when given to castrated female rats, but does not modify FSH-RF stores in castrated males (Martini *et al.*, 1968 a and b). According to these data, the ability of sex steroids to alter the secretion of the gonadotropin releasing factors appears to be linked to the sex of the animals. This indicates that the receptors for the feedback mechanisms that control the gonadotropins secretion are different in the two sexes.

Cyclicity of pituitary gonadotropin secretion: In both sexes the secretion of gonadotropins is cyclic. However, the cyclicity has different characteristics in male and in female animals.

The cyclic modifications in gonadotropins output occur in female rats every four or five days. The major event is represented by a drop of the concentration of both LH and FSH that is observed on the day of proestrus (Schwartz and Bartosik, 1962; Caligaris, Astrada, and Taleisnik, 1967; Goldman and Mahesh, 1968; McClintock and Schwartz, 1968). This drop is preceded by a similar modification of the hypothalamic concentrations of FSH-RF (Corbin and Daniels, 1967) and of luteinizing hormone releasing factor (LH-RF) (Ramirez and Sawyer, 1965, 1966).

On the contrary, in the male, pituitary FSH and LH content exhibits a cycle with a 24-hour periodicity; a peak of the concentration of both hormones is found around 4:00 p.m. (Fraschini and Motta, 1967). It is interesting that a similar cycle has been observed in the hypothalamic concentration of LH-RF, while FSH-RF apparently follows an opposite pattern and shows a peak in the morning (Fraschini, Piva, and Martini, unpublished observations).

It is believed that the cyclicity of gonadotropins secretion found in females is regulated mainly by a hypothalamic mechanism; the diurnal cyclicity of males is probably due to pineal factors (Motta, Fraschini, and Martini, 1967; Fraschini, Mess, and Martini, 1968a; Fraschini, Mess, Piva, and Martini, 1968b). It is interesting that apparently the pineal (diurnal) mechanism is present also in the female, even if the hypothalamic one is normally predominating over it. The coexistence of the two mechanisms is proved by the fact that female rats castrated when adult lose the 4-5 day cycle and acquire the diurnal variation typical of the male.

EFFECT OF ANDROGENS ON THE NEONATAL HYPOTHALAMUS

As mentioned in the Introduction, the hormonal milieu existing during particular periods of fetal and neonatal life plays a major role in directing the sexual differentiation of the hypothalamus. Apparently the presence of androgens in the earlier phases of development is essential for establishing male patterns of hypothalamic activity. The following data support this thesis.

Castration performed in male rats immediately after birth alters the development of the central nervous system so that after puberty the hypothalamic-pituitary axis becomes able to release LH in a female way, i.e., cyclically and in amounts sufficient to induce ovulation. This is clearly demonstrated by the observation that ovulation and the formation of corpora lutea occur in ovarian tissue transplanted into the anterior chamber of the eye of male rats castrated when new-born (Harris, 1964). It is relevant that only castration performed immediately after birth is effective in inducing ovulation and luteinization in the transplanted ovary; castration performed in sexually mature rats is ineffective. These results indicate that a critical period of brain sensitivity to androgens exists (Harris, 1964).

An additional proof of the physiological role played by androgens in the sexual differentiation of the brain has been obtained using as an experimental tool the very effective antiandrogen cyproterone (1, 2α-

methylene-6-chloro-4, 6-pregnadien-17α-ol-3, 20-dione). The administration of this compound to pregnant female rats induces the development of a vagina in a high proportion of male fetuses. At the same time, the hypothalamic-pituitary axis of male fetuses acquires the ability to secrete gonadotropins according to a female pattern. After orchidectomy and transplantation of ovarian tissue, male animals born of mothers treated with cyproterone develop corpora lutea and show cyclic changes in their rudimental vagina (Neumann and Kramer, 1967).

The observation that female rats exposed during neonatal life to the influence of androgens [administered either subcutaneously (Barraclough, 1967) or directly into the hypothalamus (Wagner, Erwin, and Critchlow, 1966; Nadler, 1966)] develop a syndrome characterized by precocious puberty and constant vaginal cornification may be taken as an additional proof of the role played by androgens in directing toward a male pattern the differentiation of the immature brain. Female animals treated with testosterone during the neonatal period do not ovulate because they are unable to release LH with the cyclicity typical of normal (untreated) females. It is interesting to recall in this connection that testosterone-treated females have recently been found to possess the diurnal rhythmicity in their pituitary FSH and LH content that is usually present in normal male rats (Carraro, Caviezel, and Martini, unpublished observation).

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DISCUSSION

In reply to Dr. Hahn's question about the effect of pinealectomy in the female rat, Dr. Martini said that if the operation is performed before puberty, the result is precocious puberty and an increase in pituitary concentrations of both gonadotropins, LH and FSH. Surprisingly, if one either castrates the animals or pinealectomizes them, there is the same increase in pituitary concentration of LH and FSH. If one combines the two procedures, there is no greater increase of the concentrations of the two gonadotropins.

Dr. Jost observed that the 24-hour cycle Dr. Martini had described seemed to be similar to the circadian cycle established for corticosteroids and for several other hormones. Now Dr. Martini adds certain gonadotropic hormones to the list of those secreted in different amounts in the different hours of the day. Can that, Dr. Jost asked, be considered a kind of sexually-related phenomenon?

The diurnal cycle in gonadotropins will be found only in male animals, Dr. Martini replied, because in the normal females the circadian rhythm is shut down by the ovulatory rhythm, which is predominating. But if the female rat is castrated in adulthood, there is a return to the primordial rhythm, which is the 24-hour rhythm. In addition, Dr. Martini and his associates have new data indicating that the circadian rhythm of corticocosteroids secretion is regulated by pineal factors. Understanding Dr. Martini to have said that there was a lower content of gonadotropic hormones in the pituitaries of normal male rats in the morning than in the afternoon, Dr. Jost asked whether it would be possible to find any difference between males that had mated females during the night and the males that were isolated. Dr. Martini said that he and his associates have not studied this point. Experiments of the nature suggested by Dr. Jost should be tried, he thought.

Dr. Caldeyro-Barcia asked what is the mechanism that suppresses the circadian rhythm of gonadotropin secretion in normal females. Replying that they believe it is a hypothalamic mechanism, Dr. Martini cautioned that by "hypothalamic" he and his associates do not mean exactly that hypothalamic mechanism that is set at birth by the lack of androgens.

If castration of the female leads to appearance of the circadian rhythm, Dr. Calderyro-Barcia said, it must be the ovary that is suppressing the circadian rhythm. Dr. Martini said that he and his colleagues believe that the ovary, by a positive feedback effect on LH secretion, entertains the four-day rhythm. The ovary must be present for the four-day cycle to appear. It is quite possible that the small amounts of estrogen present during the cycle permit the hypothalamic mechanism suppressing the circadian rhythm to appear.

Dr. Money, recalling the recent work of Richard Wurtman showing that there are fibers running directly from the retina to the sympathetic nervous system and then directly to the pineal, [Wurtman, R. J., Axelrod, J., and Kelly, D. F. (1968), *The Pineal*], asked whether that would indicate an environmental control of the diurnal rhythm by light. On Dr. Martini's affirmative reply, Dr. Money asked how that fact would fit into what had just been described.

"It fits in very well," Dr. Martini replied, "because of the experiments in which the light period has been shifted. The animals on which the experiments described in the formal presentation have been performed were kept in constant light from 6:30 in the morning to 8:30 in the evening: fourteen hours of light, ten hours of darkness. If the hours are changed—lights on from 6:30 in the evening to 8:30 in the morning—the rhythm shifts. We believe that by turning on the light at a different period, we shut off the enzyme that permits the biosynthesis of melatonin and 5-methoxytryptophol. This fits very well with Wurtman's research." Another experiment, Dr. Martini said, would be one suggested by Dr. Money: taking out the cervical sympathetic ganglia and studying how this influences gonadotropin secretion. But he and his colleagues have not done it.

Dr. Frenk asked Dr. Martini's opinion of the significance of the storage of gonadotropin compounds in the pituitary. He knew that up to now there have been no procedures for measuring secretion rates that may have more significance.

"We neuroendocrinologists—I belong to this big family—have been in a bad position up to now because we did not have good methods for measuring plasma levels of pituitary hormones," Dr. Matrini replied. "These methods are absolutely necessary. We are now in a little better position because we are able to measure growth hormone and LH by radio-immunological assays. It appears that even plasma levels of LH follow the 24-hour pattern exactly as does the pituitary concentration. I wish to add that there are clinical findings indicating that FSH in the blood of the human male shows a 24-hour periodicity. This agrees very well with the older data that indicate that the secretory rates of testosterone have a 24-hour periodicity in man."

The light situation prompted Dr. Kretchmer to ask whether there is a difference in the behavior of the animals in light and in darkness that may be related to a considerable amount of endocrinologic activity rather than any specific effect. The rat, replied Dr. Martini, is much more active during the night than during the day. Therefore, he is more active when the light is turned off. During estrus, the female is more active, both day and night, than any male at any time, and much more active than she is at any other phase of the cycle. This is probably another example of the estrous cycle predominating over the 24-hour periodicity found in the activity of male rats.

Dr. Hirschhorn asked for clarification of the idea of superimposition of the estrous cycle on the diurnal cycle. "If one abolishes the diurnal cycle, let us say by enucleation, can one still obtain the estrous cycle?"

"No," answered Dr. Martini. "If you blind the female rat or put it in constant darkness, the animal will not cycle any more. Nobody has studied whether or not in the dark the female animals will acquire the 24-hour rhythm. This experiment should be done."

Dr. Kretchmer asked whether the difference in behavior is really due to the light. Is it a general spectrum of light, or are there specific spectra that will excite retinal cells, which in turn would affect the particular enzyme that is found in the pineal gland? There are no data on this in the literature, Dr. Martini said. Dr. Wurtman has recently started research to find out what kinds of light are more effective on the activity of the pineal enzyme.

If pineal melatonin acts through an action on the hypothalamus, how does it reach it, asked Dr. Jacquot. "We have implanted melatonin both in the hypothalamus and in the pituitary," Dr. Martini said. "If you put it in the pituitary directly, you do not get any effect at all. If you implant it in the hypothalamus, you get a decrease in LH secretion. Dr. Wurtman has found that melatonin is secreted into the cerebral spinal fluid. So the idea is that it reaches the hypothalamus through the cerebro-spinal fluid."

Dr. Greulich spoke of Dr. Benoit's studies on the effects of light on gonadotropins; he had found the greastest effect in the long-range spectrum in red. Dr. Jost added that, in his studies on ducks, Benoit had found definite differences. He also described something that looked strange: the direct effect of light on the hypothalamus. When the eyes were removed and a piece of quartz was placed in the orbit, Benoit obtained the direct effect of light on the nervous system, at certain wave lengths.

SOME ASPECTS OF LIVER DIFFERENTIATION IN THE PERINATAL PERIOD OF THE RAT

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The present paper is not intended to be a formal, conventional scientific article. As most of the data reported here have been published elsewhere, only the final conclusions will be considered.

We have studied some aspects of the functional maturation of the fetal liver in the rat. This organ is composed of major components, the hematopoietic tissue and the hepatocytes. Each of them has its own physiology.

HEMATOPOIETIC FUNCTION OF THE FETAL LIVER

The liver is a very important hematopoietic organ in early fetal life. In the rat fetus, this function decreases rapidly after day 18 of gestation and is much reduced at birth (Nagel, 1968). This evolution is largely controlled by the circulating corticosteroids, as shown by the following observations.

a. Stressing the pregnant mother by laparotomy or by blank adrenalectomy results in an immediate and rapid diminution of the population of hematopoietic cells at whatever stage the aggression is performed. Previous adrenalectomy suppresses the effects of the stress. Thus the maternal corticosteroids, crossing the placenta, can interfere with the evolution of the hematopoietic tissue (Nagel and Jacquot, 1968).

b. After adrenalectomy of their mother and removal of their own hypophysis by decapitation *in utero*, the headless fetuses are virtually deprived of corticosteroids. In such fetuses the involution of the liver hematopoietic tissue stops, but can be resumed by hydrocortisone administration. Therefore, the endogenous corticosteroids are also of great importance in the process studied (Nagel and Jacquot, 1969).

The preceding data are based on the evaluation of the total number of hematopoietic cells (i.e., "stem cells" *plus* normoblasts) in a given volume of liver. By involution we mean that this number decreases. During this decrease, the proportion of normoblasts in relation to whole hematopoietic population increases sharply, whereas the absolute number of normoblasts per liver volume unit reaches a maximum roughly one day after the onset of the involution and then decreases rapidly. One simple explanation is to suppose that corticosteroids have two effects: cessation of proliferation at the stem cell level and acceleration of maturation of normoblasts (Nagel, 1969). The nature of the hormonal action at the cellular level remains to be elucidated.

GLYCOGEN METABOLISM IN THE HEPATOCYTES

Glycogen metabolism is but one aspect of the hepatocyte functioning. In the rat, the liver glycogen content increases sharply after day 18 of gestation. This storage is corticosteroids-dependent: fetuses deprived of these hormones before day 18 do not accumulate glycogen in their livers (reviews in Jacquot, 1958, and Jost, 1961).

The storage is related to a parallel increase in UDPG-transglucosylase activity. This activity remains low in fetuses deprived of corticosteroids; it can be restored, together with glycogen content, by administration of cortisol. Actinomycin D suppresses cortisol action (Jacquot and Kretchmer, 1964; Jacquot, 1964; Plas and Jacquot, 1966 and 1967).

The ability to synthesize glycogen and to store it is not linked, *in vivo,* with the ability to utilize glycogen. It is well known that just after birth the fetus mobilizes its liver glycogen very rapidly. Several data indicate that in the rat this process may in fact occur before birth.

a. Starving the pregnant rat produces a marked diminution of maternal blood glucose level. In such mothers, the fetal glycemias are very low (0.15 g/l) until day 20, but reach almost normal values at day 21 (0.5 g/l) (Felix, Jacquot, and Sutter, 1969; Jacquot, 1968).

b. After the interruption of uterine blood circulation, the glycemia drops very rapidly in fetuses younger than 20 days and survival time is short; at day 20, the fetal glycemia is more stable and survival time is longer; at day 21, a rapid and sustained increase in fetal glycemia follows the interruption of maternal blood supply and the survival time is considerably lengthened (Felix, 1968; Jacquot, 1968).

c. Glucose-6-phosphatase activity of the fetal liver, necessary for the over-all conversion of glycogen into circulating glucose, is very low until day 19. At day 20, it starts to increase and reaches fairly normal levels at day 21. Starvation of the mother causes a marked acceleration of this process and doubles the 21-day level (Jacquot, 1968).

It seems, therefore, that, after having undergone a first maturation (glycogen storage) around days 18-19, under corticosteroidal control, the liver cell shows a second maturation (glycogen utilization) at days 20-21. The control of this second maturation is not yet fully elucidated. Glucagon is probably an important factor (Greengard and Dewey, 1967). The effects of corticosteroids and of insulin are more difficult to interpret (Jacquot, 1968).

Hepatocyte differentiation is also at present being investigated *in vitro*. Dr. Plas, our collaborator now working in Dr. Chapeville's laboratory, has succeeded in separating the hepatocytes from the hematopoietic cells and in obtaining pure *in vitro* cultures of hepatocytes belonging to fetuses aged 14 to 18 days (Plas, 1969). The following preliminary observations were made (unpublished data).

As soon as fixed on the collagen of the petri dishes, the cells incorporate ^{14}C-labeled glucose into glycogen; in an apparently anhormonal milieu. This occurs irrespective of the origin of the cells, although cells belonging to old fetuses incorporate more than those belonging to young fetuses.

If the culture medium is not renewed, the incorporation continues for roughly 48 hours and then subsides (^{14}C-glucose is added at time 0). Cortisol hemisuccinate adjunction to the milieu at time 0 does not alter significantly the patterns of incorporation during the first 48 hours. But thereafter its action becomes conspicuous: in cells originating from 17- or 18-day-old fetuses, no "plateauing" occurs at 48 hours and the incorporation continues at a high rate; in cells belonging to 14- or 15-day-old fetuses, a plateau occurs and lasts roughly 24 hours, and then the incorporation resumes very sharply (instead of subsiding in the absence of cortisol).

If ^{14}C-labeled glucose is added to the milieu 48 hours after the beginning of the culture, the milieu remaining *unchanged,* incorporation rapidly reaches a maximum and then subsides in cortisol-deprived medium, whereas it continues to proceed at a high rate where cortisol had been present from the beginning.

If the milieu (with or without cortisol) is changed at 48 hours and replaced by a fresh one of similar composition containing ^{14}C-glucose, cortisol effect is no longer observed.

New experiments are obviously needed before any interpretation can be made, but it seems that cortisol causes a modification in the milieu that, in turn, promotes sustained glycogen synthesis and storage.

CONCLUSION

The study of developmental processes is obviously not easy. As far as our research is concerned, it started on a mere physiological basis, but it rapidly became confronted with problems of biochemistry and cell biology. One useful aspect of interdisciplinary meetings like this one could be to help us solve these problems.

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DISCUSSION

Answering Dr. Kretchmer's question about division of the cells in the experiments just described, Dr. Jacquot said it may be that two divisions take place *in vitro* during the first 48 hours, but the timing of the process is not completely clear and deserves further study. Another thing that seems important is that *in vivo* the hepatocytes are never polyploid in the rat fetus, but become polyploid after birth. *In vitro,* they demonstrate polyploidy in a matter of 24 hours.

Because they were interested first in getting the system working, he and his colleagues had not tried experiments with inhibitors of DNA synthesis or any other inhibitors, Dr. Jacquot added.

Dr. Hahn asked whether Dr. Jacquot was sure that it is glycogen that is formed from radioactive glucose. Might it not be radioactive metabolites of glucose? In other words, might not cortisone induce glucogensis? When Dr. Jacquot replied that they put labeled glucose into the milieu, Dr. Hahn said his question was answered. "In other words, when you take out the medium you do not really known whether you are taking out a different medium from the one you put in, because a different substance might be produced by the liver." Dr. Jacquot concurred.

To Dr. Hahn's question about the blood-forming part of the liver—i.e., where the nucleated cells go when there is a decrease caused by stressing the mother animal—Dr. Jacquot replied that he and his colleagues had tried unsuccessfully, by measuring the number of cells in the blood, to find an answer to this problem. Dr. Hahn said some East German investigators had found that stressing the mother rat produced an accumulation of lipids in the liver of the fetus, which the investigators took to indicate transport from the mother to the fetus. Dr. Hahn thought it might also be due to breakdown of erythrocytes in the liver and hydrolysis of phospholipids. Dr. Jacquot agreed.

Dr. Räihä asked how Dr. Jacquot and his associates had measured the longer survival of the older fetal rats after the mother had been killed. "It is quite easy," replied Dr. Jacquot. "We kill the mother at time 0 and leave the fetuses attached to the placenta *in utero*. We open the uterus quickly and take the first fetus, make a blood collection for determination of glucose, wait a minute, take the next one, and so on as long as they are alive." The fetuses were considered alive if their hearts were beating and they moved when they were pinched.

It seemed to Dr. Räihä that the younger fetuses, having a lower consumption of energy, should survive longer in complete anoxia. Dr. Jacquot replied that though he could not explain it, it does not turn out that way. To Dr. Räihä's question as to measurement of the lactate production, Dr. Jacquot answered that he and his colleagues had not studied that problem. "If you measure the blood sugar by reduction, not a specific glucose method," he said, "you probably will not obtain the results described. The reducing power of the fetal blood is not affected that much by the death of the mother, which would mean that they replace their glucose by lactate. But measuring it was not my goal. I only wanted to see if the liver would furnish glucose."

Dr. Caldeyro-Barcia asked what was the concentartion of glycogen in the liver of the fetuses in the experiments in which the mother was fasting and there was a low level of glucose in mother and fetus. Dr. Jacquot replied that it was normal despite the 72 hours of starvation of the mother. It continued normal to day 20, but at day 21 it was below what would be expected in the fetuses from a normal mother on a normal diet. This means that the processes for storage were active and would enhance glycogen deposits despite the very low glucose level. Dr. Jacquot understood this to mean that the intensity of glycogen storage in the fetuses is such that there is a permanent tendency toward hypoglycemia. This tendency is counterbalanced as soon as they are able to reutilize part of the glycogen stored. Moreover, the fetuses have an insulin level that differs according to whether the mother is nourished or not. Therefore, at day 21 they are able to deal with the situation almost as well as an adult.

Dr. Hirschhorn expressed concern about the use of the term "induction." He suggested that, in relation to mammalian differentiation, it might mean the induction of a cell type to make a certain product rather than the induction of the product. For example, cells that make adult hemoglobin and those that make fetal hemoglobin are quite different. The fetal hemoglobin-producing cell which also makes fetal carbonic anhydrase, has a different antigen on its membrane. It is a different cell. A different cell is being induced, not a change from fetal to adult hemoglobin.

"I do not believe, at least *in vivo*," Dr. Jacquot said, "that this induction is a selection of a new type of cell. We have many indications against it. Among them is that the cellularity of the liver as far as hepatocytes are concerned is not affected by the lack of corticosteroids. Once you give corticosteroids you do not see any acceleration of the mitosis, but you do see a reaction in nucleoli of cells that were there prior to injection of corticosteroids."

"That may well be," said Dr. Hirschhorn, "but you may be inducing these cells not to make a specific product that you are measuring, but to make a substance that will induce new mitosis and a change. You can do this in various culture systems."

An indication of the frequency of the mitosis in a normal liver, Dr. Jacquot said, is that the density of hepatocytes per unit volume of the liver does not change as the liver grows. One must assume that this growth is exactly proportional to the number of mitoses. With this assumption, he and his colleagues have also assumed that between day 18 and day 21 there should be not more than two cellular divisions—one every 48 hours or so. They get an effect *in vivo* with hydrocortisone in six hours. Dr. Jacquot is, therefore, convinced that it is the very same cell that is ready to react. The cell is probably not induced, but "de-repressed."

Dr. Jost added support for what Dr. Jacquot had reported. "When one stops glycogen storage with the method Dr. Jacquot used, and then induces it by injecting cortisone in the fetus, if one looks at these hepatocytes thirty minutes after the injection, one hour, two hours, four hours, and so on, the first thing to happen, we noticed, is incorporation of labeled uridine into the nucleoli, which happens after approximately thirty minutes. Then there is an increase in size and a change in shape of the nucleoli. The increase in glycogen in these cells begins after twelve hours. We studied this stage by stage, and we did not see any mitosis."

PRIMATES

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INTRODUCTION

In a multidisciplinary undertaking such as this symposium, said Dr. Hamburg, one of the dilemmas is to achieve technical depth and mastery in highly specialized areas by scientists who also have at least some acquaintance with the concerns of other disciplines, some reasonably close to their own and others at some distance. To do this calls for an ability to recognize the frames of reference of other disciplines concerned with so broad a common interest as sexual differentiation. Following presentations of research at the molecular level of organization, the planners of the symposium have proceeded to social and psychological levels. The disciplines involved at these levels, having for the most part only recently been generally recognized as scientific, are at an earlier and perhaps more critical period of development than are those of the biological sciences; but the problems they deal with are of inestimable importance for all mankind.

ENVIRONMENTAL INFLUENCES ON SEXUAL DIFFERENTIATION

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Primate societies are semipermanent organizations in which members have specific social roles (Bernstein and Sharpe, 1966; Gartlan, 1968). Many of these roles are typical of males and others of females. For these roles the quality of the environment is an essential determinant. Field and laboratory studies have shown that environment strongly influences several aspects of sexual differentiation. This report reviews existing knowledge and illustrates a unique experimental approach to the analysis of social interaction developed in our laboratory.

MATERNAL ROLE AND ITS EFFECT ON SEXUAL DIFFERENTIATION

Although maternal care is basically similar among primate species, its duration varies greatly, being longest for the most advanced nonhuman primates, the chimpanzees and gorillas. The length and quality of maternal care vary according to the sex of the infant.

In our laboratory studies of early mother-child interactions in the pigtail monkey, we found a number of ways in which mothers treated their male and female offspring differently (Jensen, Bobbitt, and Gordon, 1968a). In the early weeks of life, mothers and their male infants separated more often; mothers became increasingly punitive, carried, cradled, and restrained them less. Mothers of males directed more of their own total behavior toward the environment. No sex differences were found in infants' behavior toward their mothers in this early period of life. The differences in maternal behavior suggest that the mother plays a role as instigator of the greater independence that is typical of males. Similar results have been obtained with rhesus monkeys (Mitchell, 1968a). Mothers in this study touched, groomed, and restrained female infants more and moved away from, played with, and presented to male infants more often than they did with female infants. Male and female infants under three months of age did not differ in behavior toward the mother. Male and female infants between three and six months of age differed significantly in one type of behavior toward the mother—the males pulled and bit their mothers more. This finding supports general observation that male infants are rougher on their mothers than are females.

The length of time that offspring associate with their mothers varies with sex. In naturally-living macaque monkey groups, male offspring leave the mother at about three years of age and thereafter seldom associate with her. On the other hand, females remain close to her for many years, even into reproductive adulthood and sometimes for life (Sade, 1965). This is particularly striking in Japanese macaques; one sees subgroups of kin consisting of a grandmother with several female offspring, each of whom in turn is surrounded by several of its offspring.

MALE ROLE AND PATERNAL BEHAVIOR

In naturally-living macaque and baboon groups, the leader is the most dominant male. His characteristic functions include controlling the movement of the group, defending the group from outside threats (often with the help of other high-ranking males), and protecting group members from each other by intervening in fights. He does most of the mating himself.

In our captive group of pigtail monkeys, we saw how the group functioned under two different leaders. The first leader was relatively ineffectual; he did not interfere in fights and chases among group members. One infant was killed and another was seriously injured from continual chasing by older monkeys. One month after we formed the group, the original leader was overthrown by the second-ranking male. The new leader proved very competent. He performed all the behavior characteristic of his role. After the group stabilized, we did an experiment to test the hypothesis that the leader reduces the level of aggression in the group (Tokuda and Jensen, 1968). We removed the leader temporarily and found that the amount of aggression among the remaining group members increased significantly. When we removed other high-ranking members, there was no change in the level of aggression.

Hamadryas baboons of Ethiopia maintain stable units composed of one adult male, the leader, and a harem of several females (Kummer, 1968). The leader male herds the females together and they are not allowd to leave him. Communication between the different units is performed mainly by the leaders. Young adult males are protective and paternal toward the female infants and juveniles, but eventually are excluded from the parent unit. Later they take on young females from any unit. Kummer believes that the paternal behavior by young adult males toward young females may be an important determinant of the one-male group structure typical of this species.

As yet there are no experimental studies of the development of the leader in any species. It seems likely that this role is the culmination of a continuous process of learning by individual members of an established group. Although unknown, it is possible that the striving for leader role has some genetic basis. Some monkeys appear to want to be dominant over all others. We noticed in our captive group of pigtail monkeys that when a monkey became leader he appeared equipped for the role, although the behaviors characteristic of a leader had not been evident in him before. What makes a leader seems to be a combination of determinants. Leaders are usually large and aggressive, have a confident striding gait, and are able to control the group. If any single factor determines the role it is still unknown. Having a mother of high rank may be important, since it has been observed in rhesus (Koford, 1963) and Japanese macaques (Kawai, 1958) that male and female offspring attain a dominance rank comparable to that of their mothers. Sons of high-ranking females remain in the central subgroup, which consists of high-ranking males, females, and infants. When sons of low-ranking mothers are two to three years old, they go into the peripheral subgroups of lower-ranking male monkeys.

Paternal behavior in nonhuman primates is more common than has been generally believed (Mitchell, in press). In many species in the wild, particularly Japanese macaques (Itani, 1963) and baboons (Mitchell, in press), males may exhibit maternal-like behavior toward infants or juveniles. They cradle, retrieve, and carry the infants just as the mothers do. One study showed that early environmental deprivation profoundly destroyed normal paternal behavior patterns of rhesus monkeys (Mitchell, 1968b) just as it does maternal behavior, as will be discussed later.

ISOLATION AND DEPRIVATION EXPERIMENTS

Environmental variables can be identified, isolated, and accurately measured in the laboratory. Several excellent studies on primates show the environmental effects on the development of sexual behavior and sex role. Rhesus monkeys raised in social isolation for the first 28 to 29 months of their lives failed to develop normal sexual behavior (Mason, 1960). Deprivation of all social relationships (both mother and peers) in these first years totally destroyed their ability to mate normally as adults. Males appeared to suffer greater damage than did female (Senko, 1966), and intensive rehabilitative efforts were to no avail (Harlow, 1965). Our studies revealed that male infants raised by their mothers in a privation environment showed more deviant behaviors than did females raised in the same situation. The males sucked their thumbs more, bit their mothers more often, and did more upright walking (Jensen, Bobbitt, and Gordon, 1966). Findings from all these experimental studies suggested that to develop healthily without deviant behaviors, male infant monkeys need a rich environment more than females do.

Several experiments have shown that normal maternal behavior in monkeys is dependent on early environmental experience. Female rhesus monkeys separated from their mothers at birth and raised in social isolation for the first two years of life developed into totally inadequate mothers (Harlow, Harlow, Dodsworth, and Arling, 1966). They were brutal toward their newborns, particularly males. They persistently rejected and sometimes even killed them. Male infants of these mothers grew up to be extremely aggressive, much more so than females (Mitchell, Arling, and Moller, 1967). It will be revealing to know how this rearing experiences will affect the females when they grow up and become mothers.

All studies of early social isolation indicate that the earlier and more severe it is and the longer it lasts, the more devastating its effects. For normal social, sexual, and maternal behavior, the earliest developmental period of life appears to be crucial. Later in infancy, three to nine months in rhesus monkeys (Clark, 1967) or in the second year for rhesus monkeys (Joslyn, 1967) and chimpanzees (Davenport, Menzell, and Rogers, 1966) social isolation has little effect.

It is clear that sexual behavior is irreversibly crippled by inadequate early experience. However, peer experience early in life, even in the absence of the mother, prevents the development of abnormal sexual behavior. Rhesus monkeys exposed to playmates for as little as 20 minutes a day in the early months of life developed adequately both socially and sexually (Harlow and Harlow, 1962). The variables in peer play that facilitate normal development of sexual behavior remain to be defined.

One current study is designed to test the effects of a moving object that simulates a playmate (Mason, personal communication). Infant rhesus monkeys are raised without mothers or peers but with a moving surrogate—a terry-cloth-covered plastic jug set on a pie pan, which moves around in a circle and up and down at random intervals. The infants develop an attachment for it similar to that described for stationary surrogate mothers (Harlow and Zimmerman, 1959). Its movements provide the infant with an opportunity to engage in chasing and active kinds of behavior. Although incomplete, the study already shows that these infants demonstrate far fewer stereotyped and autoerotic behaviors characteristic of those raised on stationary surrogate mothers (Harlow and Harlow, 1962).

A privation environment is not exclusive to the laboratory. Natural habitats of some species of monkeys vary widely from lush forest to dry open grassland with a sparse food supply. With these differences in ecology there are marked differences in the sex ratio of the groups, in social organization, and in the mother-infant bond. Males and females are about equal in African green monkey (*Cercopithecus aethiops*) groups (Gartlan and Brain, 1968) and in baboons (Rowell, 1966) living in forest areas where food is plentiful. Groups of these same species living in sparse open grassland have a preponderance of females. African green monkeys in the latter setting show stronger mother-infant bonds, which may be of considerable adaptive significance in these areas where predators are more common than in the forest. The discovery of a number of such intraspecific differences has led to the suggestion that social structure in many primate species is habitat- rather than species-specific (Gartlan, 1968).

While many laboratory and field studies show that environment definitely plays a role in sex differentiation, it would appear that innate factors are important as well. If rhesus monkeys are raised in complete isolation, there is no chance that the mother can convey sex role to the infants. In experiments of this kind it was found that female infants were more passive, seldom initiated rough-and-tumble play, showed less frequent threat responses, and did not threaten males in contrast to males who threatened both sexes (Rosenblum, 1961, and Harlow, 1965). The hormonal influence on this kind of behavior of young monkeys has been shown by Goy (1966), who injected pregnant monkeys with male sex hormone, producing female pseudohermaphrodites. These female infants behaved more like males.

PATTERNS AND SEQUENCES OF SOCIAL INTERACTION

To investigate the effects of environment on sex differentiation, we have studied mother-infant interaction in pigtail monkeys living in environments with different amounts of social and material stimulation (Jensen and Bobbitt, 1968). Mother-infant pairs were raised from birth in isolation in individual barren cages in sound-proof rooms. We called this a privation environment. Other mother-infant pairs were raised in identical cages with climbing opportunities, numerous toys to manipulate, and in the open laboratory where they could see and hear other monkeys and people. We called this a rich environment. We wanted to know the effects of these two different environments on the development of social behavior.

Our method and experimental design have been reported (Bobbitt, Jensen, and Gordon, 1964), but in brief were as follows. The subject in this study were 14 mother-infant pairs of *M. nemestrina.* Nine pairs (six females and three males) were placed in individual cages (4 ft. by 4 ft.) in the privation environment. Five pairs (three females and two males) were placed in the rich environment.

The behaviors occurring during a daily ten-minute period were recorded in detail. We defined about forty units of behavior, each qualified by who did it, toward what or whom it was directed, and the positions of the subjects in relation to each other. Observations were recorded on a standard tape recorder. A coding system in which a letter or number was assigned to each behavioral unit and its qualifiers permitted the rapid recording of each event in sequence. Duration of behavior was measured by three-second intervals on the tape. Interobserver reliability was maintained at over 80 percent.

Of the four positions we defined for observation, the most physically separated, position 4, defines our concept of independence.

Early in life males and their mothers spend more time in the most independent position than do females.

Monkeys in the rich environment were more independent than those in the privation environment (Jensen, Bobbitt, and Gordon, 1968). This is shown by time spent in position 4. The developmental trend for infants in the two contrasting environments is shown in Figure 1. A female infant raised in a privation environment was the least independent of all infants.

As a further test of the effects of environment, we separated the infants from their mothers at six months of age and paired them for dominance testing (Jensen, Bobbitt, and Gordon, in manuscript). Males raised in the rich environment were dominant over all others. Females from the rich environment were dominant over privation males. The least dominant infants were the females raised in privation. These findings suggest that the privation environment not only affects an infant's independence from its mother, but also tends to make it less socially adequate in competing with peers.

We are investigating how the environment effects these differences in independence. We have hypothesized that there would be changes in the interactive relationship between mother and infant. We approached this by an analysis of sequences of behavior. We developed a unique computerized method to analyze patterns and sequences (Bobbitt, Gourevitch, Miller, and Jensen, 1969). This approach gives a better

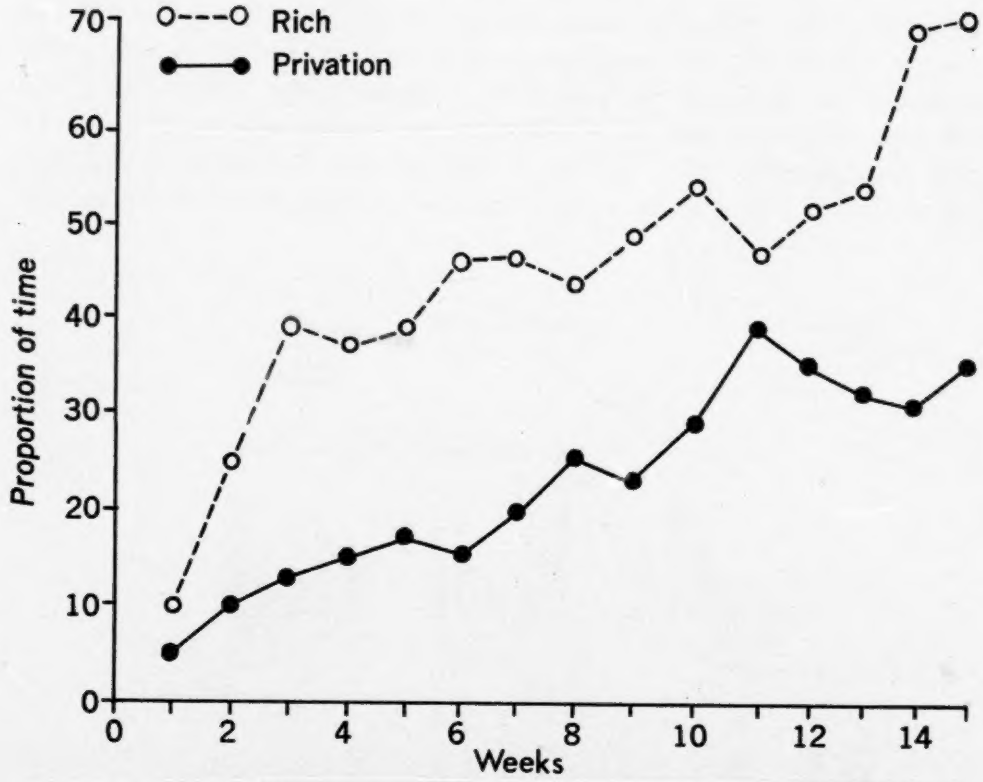

FIGURE 1.—Proportion of total time mothers and infants spent separated.

understanding of the dynamics of social interaction than does the customary analysis limited to frequency data. We are currently analyzing the sequences of several behaviors involved in independence. These include nursing, mother leaving infant, infant leaving mother, thumbsucking, cradling, and punishment.

To do sequence analysis we first analyzed our data for behavior patterns. A behavior pattern was defined as a behavioral unit and its qualifiers or any number of simultaneously occurring behavioral units. Special adaptations of chi square statistics were used to determine significant patterns ($p < .01$). Sequences were determined by a Markoff chain analysis of significant patterns. This procedure yielded the conditional and unconditional probabilities for each succedent pattern and determined their significance ($p < .05$) (Bobbitt, *et al.*, 1969).

We have completed an analysis of hitting behavior of mothers to test the following hypotheses: through punishing behavior mothers play an active role in the development of infant independence; punishment by the mother causes an infant to leave her.

We found that a mother hit her infant primarily when it was either climbing on her or in some way was active and touching her. To test the hypothesis that hitting functions to increase the probability that the infant will leave the mother and stop climbing on her, we examined the sequences of behavior patterns that followed a hit (Figure 2). The consequences of a hit were different for the female infants in the two environments. Most of the females in the rich environment and the males in either environment left the mother, but females in the

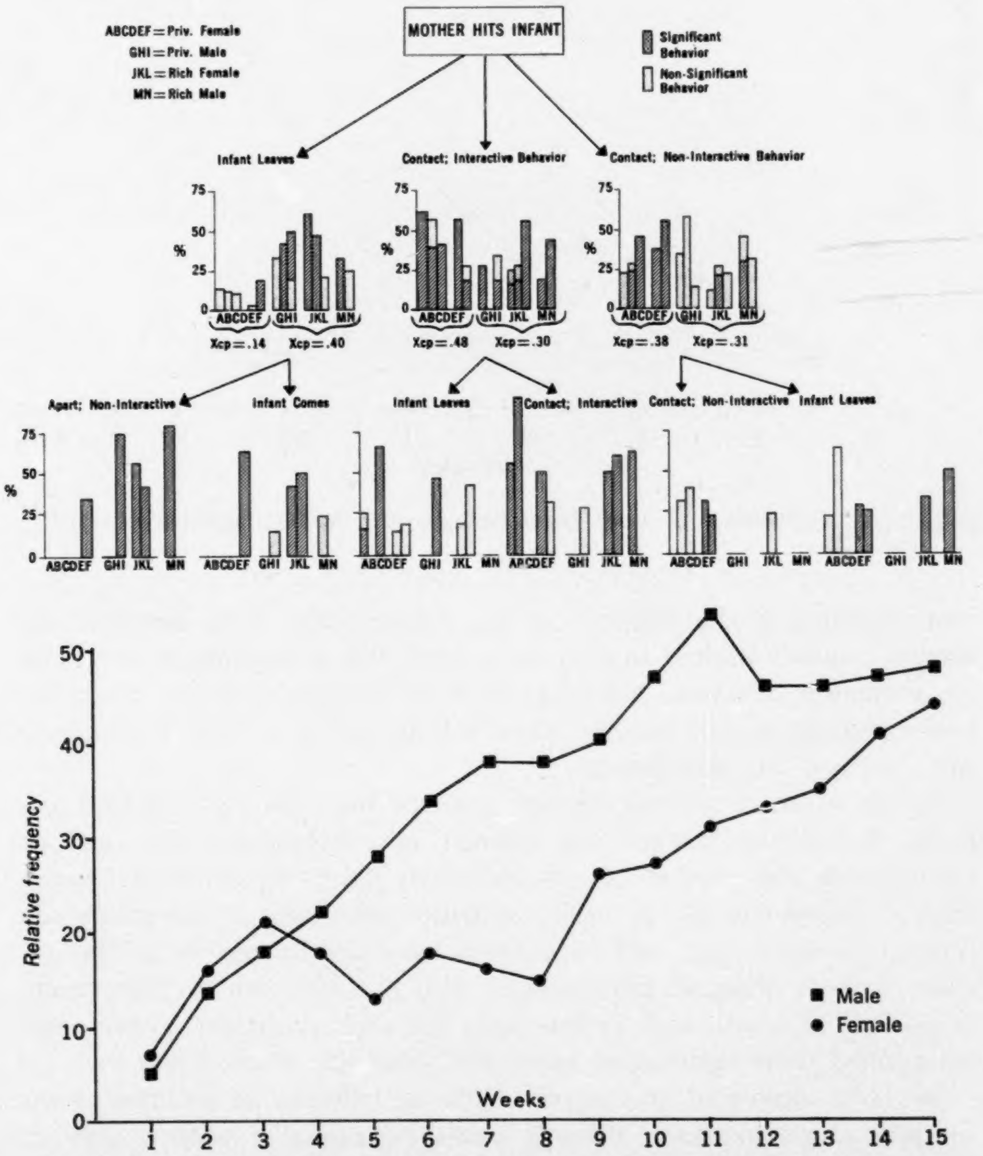

FIGURE 2.—Immediate succedents of mother hitting infant for the 14 mother-infant pairs. Bars designated partly significant indicate several different patterns under the general group heading, one of which was significant and others not significant.

privation environment did not leave. (In only one of the six privation females was leaving the mother a significant immediate succedent to the mother's hit.) The mean conditional probability of leaving the mother was .14 for privation females compared with .40 for the combined group of rich females, rich males, and privation males. The privation females were most likely to remain in contact with her and to engage in either interactive behavior (e.g., cradling, nursing, climbing, hitting again, or punishing) or noninteractive behavior (e.g., handling self or cage, grooming self, changing posture). These results are consistent with the finding of less independence in privation females. They also support the hypothesis that punishment is one means by which mothers instigate independence of the young.

When we analyze other sequences of behavior we expect to find a number of other disruptions in the behavioral contingencies of the mother-infant relationship effected by environment. Environmental effects may well be manifest in the disruption of a number of the sequences of interactive behavior. All these changes will provide a meaningful explanation of effects and will suggest hypotheses for subsequent experimental tests.

In summary, sex differentiation in primates is severely disturbed by environmental deprivation in the earliest months of life. We do not yet know what constitutes the minimal maternal care or peer access necessary for normal social and sexual behavior development, or why an enriched environment is beneficial. Males appear to be more vulnerable to deprivation, suggesting that normal male sex differentiation is more delicately balanced. In some species of primates, mothers, adult males, and juvenile males treat an infant differently depending on its sex. Local ecology has significant effects on social structure and on behavior in some primate groups.

Our knowledge of environmental sex determinants is scattered over a variety of species of primates. We must be cautious in generalizing from one species of primate to another. Improved methods for observing and analyzing primate behavior will provide a better understanding of social development. If these methods are applied in the field as well as in the laboratory, results can be better integrated.

Primate studies indicate that normal sex differentiation, the kind that makes possible the complex social structure of primates, does not occur without the shaping forces of the environment. These forces are multiple and so are their effects. We cannot generalize from primate studies to human beings, but a knowledge of the basic processes of primate social interactions can suggest ways of investigating parallel human interrelationships.

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SEXUAL DIFFERENTIATION AND THE EVOLUTION OF AGGRESSIVE BEHAVIOR IN PRIMATES

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There has been a great upsurge in research on the evolution of human behavior in recent years, the result of much new interest, new information, and new ideas on an old subject. Among the many sources of information are the fossil record, prehistoric archeology, chemical dating methods, study of the few remaining hunting-and-gathering societies, and especially the study of nonhuman primates, chiefly old-world monkeys and the great apes, animals with fairly complex patterns of living.

Despite the antiquity of the subject matter, only recently has there been scientific investigation on any substantial scale. Hence, generalizations are tentative; but the viewpoint is fundamental in efforts to understand the nature of man.

Keeping in mind the general time-scale of evolution may help to point up a dilemma in our current situation in human biology and behavior. There have been mammals on earth for well over fifty million years. Primates appeared early among the mammals; they have been present for many millions of years. We have recently learned that a distinctly manlike form has been present for about two million years—possibly more. He was manlike in the sense of standing upright, although his brain was much smaller than that of contemporary man. Our own species has been in existence for about forty to fifty thousand years. Agriculture has been with us perhaps somewhat less than ten thousand years; the industrial revolution, of course, began about two hundred years ago.

Problems that concern us so urgently today—the enormous population growth, urbanization with its difficult consequences, a physically sedentary way of life, and at least an attempt at a worldwide community—are largely products of the industrial revolution, indeed, chiefly of the past few generations. We see, then, that some of the chief features of the contemporary environment are products of the most recent phase of evolution, much of which has taken place within our lifetime.

About a decade ago, Henry Nissen wrote: "The behavior of animals is a major contributing factor for their survival, and consequently, through the mechanism of heredity, for the course of evolution. Maintaining favorable relations with the environment is largely a function of behavior. Possessing efficient skeletal, circulatory, digestive, sense-organ, and effector systems is not enough. All these must be used effectively in activities such as food getting, reproduction, and defense. Behavioral incompetence leads to species extinction as surely as does morphological disproportion or deficiency in any vital organ." (Nissen, 1958).

Animal behavior may be investigated in the laboratory, as Dr. Jensen has indicated, in artificial colonies, or in natural habitats. The kinds of information we get from these different approaches are largely complementary, and all are necessary if the complex roots of behavior are ever to be understood. Clearly we are only at the early stage of such investigations. The field studies in the natural habitat are extremely helpful in understanding the way that structure and behavior are adapted to environmental conditions. It is really the adaptive aspect that makes these studies interesting; they tend to stimulate laboratory investigations in which relevant variables can be controlled.

In the past few years there has been strong interest in the possible evolutionary roots of human aggressive tendencies. There have been some provocative findings such as those touched on by Dr. Jensen and others in this symposium. For example, there is the very early sex difference in aggressive behavior that has been described by Rosenblum (1961) and confirmed by others. Male infants engage in social threat and in rough-and-tumble play much more than female infants do. As Dr. Jensen has noted, the males bite their mothers more in the early weeks of life. This early sex difference probably has an important hormonal mediation, as has been indicated repeatedly here. In monkeys, the work of Young, Goy, and Phoenix (Goy, 1968) has shown the way in which androgen administered to the pregnant mother will shift the female fetus in a malelike direction both anatomically and behaviorally. There are quantitative data showing that several kinds of aggressive behavior—particularly social threat—are shifted in the male direction, a change that has persisted into adult life under the laboratory conditions in which it has been studied.

In field studies, Hall and Devore (1965) have shown a persistent difference between males and females in aggressiveness of play. Especially among the species that spend much time on the ground, the males play very aggressively early in life and through the years of growth and development. When they come to adulthood, the adaptive utility of their skill in aggressive interaction becomes apparent as they cope with predators, the great carnivores of the African plains. An interesting observation of the past decade is that social organization functions as biological adaptation in the sense that males defend the group. In many baboon troops, though not all, when a predator appears the main body of the troop will head for the nearest trees as fast as they can, while one or more adult males hold their ground. Soon a phalanx of adult males with large canine teeth and massive temporal muscles and very aggressive dispositions is interposed between the predator and the others in the troop.

One of the important outcomes of the sex differentiation process in the higher primates, then, lies in the direction of male aggression, mediated in part by hormonal factors prior to birth and augmented by experience for years after birth.

Despite recent interest in the subject, very few field studies of primate behavior have focused primarily on aggressiveness (Washburn and Hamburg, 1968). Therefore, my son and I began a field study of baboons and chimpanzees in East Africa last year, centering our attention on aggressive behavior in the two species. We were fortunate in getting more than 200 hours of observations of animals within ten yards of us. We also had generous access to the files of the unique chimpanzee study in Tanzania conducted by Jane Goodall from 1960 to the present. With help from her and from Phyllis Jay in Kenya, we acquired a good deal of data on aggressive behavior in the two species. We hope to extend these observations in future work.

By "aggressive behavior" I mean behavior in which an animal inflicts, or tries or threatens to inflict, damage on another animal. We are, in other words, concerned with threat and attack patterns. We try to describe such patterns, to determine the conditions under which they occur and the circumstances in which these patterns are likely to be diminished or terminated, particularly by means of inter-animal communication.

We choose chimpanzees because they are probably mankind's closest living relatives; we and they are descended from a common ancestor who lived some millions of years ago. There are many similarities between man and chimpanzees in the number and form of chromosomes, in blood proteins, in immune responses, in brain structure and behavior. The more we see of their behavior, the more we are impressed with the uncanny resemblance between some of their communicative repertoire and that of the human being.

This is not to imply that we inherit immutably fixed action patterns. The chimpanzee's adaptation depends heavily on learning, and ours does so even more. It is important, however, to consider whether learning may be easier for us in some areas than in others. Language provides a good example in this context. The content of any language is learned; but the students of child development are increasingly interested in the possibility that there is something about the circuitry of the human brain that draws the infant toward language experiences. Our question is: Has nattural selection operated on early interests or preferences so that the attention of the developing organism is drawn more to some kinds of experiences than others?

A model for studying this question is suggested by an experiment of Gene Sackett's from the University of Wisconsin (Sackett, 1966). Using the technique of rearing-in-isolation described by Dr. Jensen, Dr. Sackett presented to the total isolation-reared infant, a number of different stimuli, such as pictures of other monkeys in various situations and a variety of non-monkey pictures. He was able to measure several kinds of responses in the infant monkeys. He noted first of all that their interest was drawn more to monkey pictures than to those not showing monkeys. Secondly, at about the age of two and a half months, the infants raised in total isolation showed enormous interest in the full-face threat of another monkey. Apparently there is something in the brain of the Rhesus monkey that calls forth this response.

If these results hold up on replication, they suggest that, once attention is drawn to such a complex stimulus, a great deal may be learned about the conditions under which this kind of behavior is appropriate, when it occurs, what stops it, and so on. Much learning about aggressive behavior might follow from that simple interest in this kind of stimulus.

In field and experimental studies there is considerable evidence of learning in regard to aggressive behavior. There is no reason to think that most of it is essentially reflexive in the higher primates. In all of the nonhuman primate societies, especially the ground-living ones, aggressive behavior is present in abundance, with many similarities in its expression and in the contexts of its occurrence. But there are variations in keeping with local conditions, even within a species. So perhaps it has been adaptive in evolution to learn easily when and how to be aggressive, with flexibility in keeping with environmental conditions.

We observed a community of about fifty chimpanzees living in an area of about ten or twelve square miles of moderately dense forest. Bananas, a premium food for both chimpanzees and baboons, were available in the area. The pattern of social organization of the chimpanzees was fairly fluid.

In the next few paragraphs, I wish to sketch briefly some observations we made on chimpanzee and baboon aggressive behavior. Similar behavior sequences have been carefully described by Jane Goodall (Goodall, 1968) and richly photographed by Baron Hugo von Laurick.

On one occasion we watched a highly dominant adult male chimpanzee sitting right next to a baboon that was giving a major threat. Another powerful adult male baboon was nearby. The chimpanzee, the most dominant of his community, did not respond to the baboon's threat. In general, the chimpanzees were dominant over the baboons.

Dominance is a striking feature of chimpanzee and baboon life. The dominant male chimpanzee had a bunch of bananas and was taking a little rest, with three powerful male baboons sitting around him. They stayed close to him, keeping the pressure on for over an hour. Eventually the chimpanzee sat up and ate a banana. Another dominant chimpanzee also ate some bananas. The baboons got only the peelings. As the baboon harassment continued, the chimpanzees became a little tense; one picked up a bunch of bananas and sat on an elevated platform. Soon one of the baboons broke off the rather tense interactions with the male chimpanzees and attacked a female chimpanzee. She climbed a tree, striking down at the baboon as he chased her up the tree. This redirection of aggressive behavior is common. In a tense interaction, one animal will break off and attack a smaller, more vulnerable animal. Such occurrences are common. Sometimes one vulnerable animal appears to be a consistently preferred target.

Another bit of aggressive action came about when a low-ranking chimpanzee who was a skillful thrower threw a stone quite accurately, hitting one of the baboons, who ran away. Another baboon came up to investigate, and was promptly attacked by the more dominant baboon. Again there was redirection of aggression from the more to the less dominant.

There is a rich repertoire of signals among chimpanzees that have the effect of turning off aggressive sequences. These actions only alter probabilities; none will work absolutely and uniformly, contrary to what has been written by some students of animal behavior. It is also not true that these animals never injure each other. One of the calming signals is the touching of the scrotum of the aggressive animal. But on one occasion we saw an adult female chimpanzee reach behind an adult male who was gathering up bananas after there had been some fighting and cautiously touch his scrotum. It didn't work. He attacked her. An infant watching the scene ran to his mother, who beckoned with outstretched arm (in any fearful situation the instant reunion of mother and infant is impressive); and another adult male fled about forty feet up a tree and stayed there for some minutes. Another response is the typical fear-face expression, which has the communicative value of recognizing dominance and tending to diminish aggressive behavior on the part of the more dominant animal.

Chimpanzees acquire a good deal of skill in the use of tools. We have observed a young chimpanzee about four years old (chimpanzees are not fully mature until about the age of ten or twelve years) attack an adult male baboon with a ten-foot palm frond, driving him off into the forest. The young chimpanzee had watched two older siblings engaging in much the same kind of behavior.

The chief mode of learning for the nonhuman primates is a sequence that goes from observation to imitation, then to practice. They have full access to virtually the whole repertoire of adult behavior with respect to aggression, sex, feeding, and all other activities. The young observe intently, and then imitate, cautiously at first, all the sequences they see. Then they may be seen practicing these sequences minutes or hours after they have occurred. This observational learning in a social context becomes exceedingly important for the young primates. It takes the place of active instruction on the part of adults, which seems never to occur.

Male chimpanzees and baboons, even in infancy, go through the aggressive patterns much more than the females do. Our observations on this point are entirely consistent with those of earlier workers. Also the protection of infants by both sexes elicits vigorous aggressive action. The female shows a striking change in aggressiveness when she has an infant.

In briefly sketching an adaptive evolutionary view of aggressive behavior, I have touched on some of the ways in which aggressive behavior may have functioned adaptively through the long course of evolution, 1) in increasing the effectiveness of defense, 2) in providing access to valued resources such as food and water, 3) in contributing to effective utilization of the habitat by distributing animals in relation to available resources—a spacing function of intergroup tension—and 4) in resolving serious disputes within a group and providing a predictable social environment.

Factors of this sort may have given selective advantage to aggressive primates, providing they could manage effective regulation of this aggressive behavior. They have well-defined cues that usually terminate aggressive sequences. They have an elaborate repertoire of submissive behavior. They have a stable, agreed-upon dominance hierarchy that contributes to the predictability of the social environment, and they have vivid aggression-submission-reassurance sequences that have many elements in common with the behavior of man. I think future research will profit from those who pay as much attention to the regulation and control of aggressive tendencies as to their instigation.

Behavior that has had selective advantage for millions of years is likely to be a part of the human heritage, transmitted both biologically and socially. It is difficult for organisms to change rapidly when the environment changes rapidly. Such environmental changes have often been associated with extinction of species. Yet the rates of change associated with extinction are usually slower than those we now experience.

If we are going to adapt to these unprecedented changes in environmental conditions brought about by industrial technology and its biosocial consequences, we shall have to understand much more about the nature of the human organism, the main forces that shaped it through its evolution, and the ways in which these recent environmental changes impinge on the very old equipment—genes and customs—that we bring with us.

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DISCUSSION

Dr. Jensen's use of the word "vulnerability" in relation to the male interested Dr. Money. He thought great attention should be paid to that point in all studies of human beings. The conception rate for males is much higher, 120 to 140, some people have estimated, as compared with 100 for females. The birth rate is 106 to 100 females. The danger of imperfect sexual differentiation appears to be greater in the male than in the female, male differentiation always requiring that something be added over and above that required for female differentiation which takes precedence. All the psychosexual anomalies are substantially greater in the male; some of them—the most bizarre ones—are unheard of in the female.

With respect to the gender-identity disorders, particularly feminine homosexuality, Dr. Money continued, he wondered whether there might be some deficit in the aggression factor discussed by Dr. Hamburg in these people, so that they are not able to enter into the competition of ordinary little-boyhood.

Dr. Money also asked Dr. Jensen and Dr. Hamburg to consider the question whether it would be a good idea if we as a species followed the other primates and allowed more overt sexual play in childhood as a guarantee of psychosexual normality.

Referring first to the point about male "vulnerability," Dr. Jensen said male monkeys are more fragile than females in several respects. However, compared to man, primates do not seem to have a great variety of sexual disorders, so far as he knew. Dr. Money said that a recent article on chimpanzees indicated a great resemblance to human experience in that species (Kollar, E. J., Beckwith, W. C., and Edgerton, R. B. (1968). *J. Nerv. Ment. Dis.* 147, 444-459.) so much more like human beings than monkeys are, Dr. Jensen remarked, that the more we learn about them the more surprised we are at their resemblance to us.

Dr. Jensen felt it would not be desirable for professionals to encourage parents to allow their children to engage in more overt sex play. He agreed that it would *seem* healthier to allow sexual behavior development to proceed freely, as it does among the primates; however, in our society the great problem is that the attitudes of adults would have to change along with the behavior they would permit. Most parents today feel uncomfortable and even guilty when just discussing sex with their children. When parents do not feel natural about something, they often go to extremes. Dr. Jensen was reminded of the extremes that parents and educators went to some twenty years ago when they were advising permissiveness in many kinds of behavior. While this was based on some rather sound ideas of the psychoanalytic movement, the attempts to implement it produced some undisciplined problem children. He thought that professionals should assist parents in understanding that the sexual exploration and sexual play characteristic of normal boys and girls of all ages is a natural and healthy aspect of development.

Dr. Hamburg observed that the difficulty with making any such general recommendations as engaging in more overt sexual behavior is that there is a social system with interrelated parts. Modifying one part might produce unanticipated ramifications in another part.

From the evolutionary point of view, he continued, the lack of access that the young have to the full range of adult behavior is a recent development. Dr. Lambo had mentioned that access, which seems to have been available in early human societies—probably the hunting-and-gathering societies of some two million years ago in human evolution. It certainly exists for the nonhuman primates in all phases of adult activity.

Sexual behavior in the chimpanzee is a striking example, said Dr. Hamburg. Parenthetically, he noted, earlier reports that chimpanzees are not very sexy are not true. They reflect the inability of observers to see what goes on in the dense forest. He had seen more copulations in a month among the chimpanzees than other studies had reported in two years. It is common for the mother's youngest infant to come right up to the copulating animals and poke around, looking and smelling, etc. Once in a while the male will push the infant a few feet away. This is the way young chimpanzees learn about sex.

The modern industrial social system has greatly changed all that, Dr. Hamburg said, not only in respect to sex, but in respect also to occupation. How does a son know what his father does when the father is a scientist attending a conference in Washington?

Dr. Williams-Ashman asked Dr. Jensen what happens to the monkeys reared in total isolation when they reach puberty. Do the females cycle normally?

Dr. Harry Harlow at the University of Wisconsin has done the principal studies of these monkeys, Dr. Jensen answered. As far as he knew, early social isolation has no effect on the monkeys' physical maturation, and the females do have normal estrus cycles.

Physiological differences between newborn monkeys, chimpanzees, or other primates and human infants are so great, Dr. Barnett commented, that studies of newborn human babies become very important. According to the data of these two speakers it had apparently been impossible to show male-female differences in the mothers' attitudes toward offspring in the early period of life. He wondered how early in the infant-mother relationship, in Dr. Jensen's and Dr. Hamburg's observations, the differences had appeared. It seemed to him that an understanding of the mechanism of the mother's role might be very important in relation to how early she distinguishes between the sexes.

Dr. Jensen replied that the chaos of the early days of the human baby's life prevents good observation of the mother-infant relationship. The mother does not usually see her baby very much in the hospital. The first week after mother and baby go home, the environment is still so disrupted that it is hard to get good measures. Dr. Kagan has said that he thinks mothers have one program for sons and one for daughters, and that they behave differently from the start. Dr. Jensen agreed. He and his colleagues have found a number of ways in which mothers treat their male and female babies differently in the first weeks of a monkey's life. For example, mothers punish male infants more than females. The differences are quantitatively small, but the trends continue and are statistically significant.

Monkey mothers apparently begin to show signs of recognition of their infant's sex on the day of delivery, Dr. Jensen continued. They are very careful in examining male genitalia as they groom their male infants right after birth. They do not examine the females' genitalia nearly so carefully. We can assume that monkey mothers know what male genitalia look like, presumably on males of any age, and they obviously look carefully at them in the first days after birth.

Dr. Gutmann asked both speakers whether they had noticed any consistent age differences between males occupying more or less the same status within the band—consistently different characteristics in modes of relatedness and behavior between old leaders and younger ones. Dr. Jensen had said in conversation that an increase in paternal behavior had been noted on the part of older fathers in primate bands; they behave toward infants much as a mother does. Have any other differences of this nature been observed?

Two such differences in the Kenya baboons had interested him very much, Dr. Hamburg replied. In a group of forty animals they were studying intensively, there were two such cases. Although he was not certain, he thought they were the two oldest adult males. In any case, each of these two males had a particular infant with him all day long. In the savanna country, with the tall grass, if one was seen it could be predicted that the other would appear in a few seconds. One of the males was clearly the Alpha male, the most dominant in the troop. He was highly aggressive in other situations, but very tender and nurturant with this one infant. The attachment between the two was so close that there seemed to be no knowledge of who was father in these relationships.

Dr. Jensen added that similar relationships have been observed in the Japanese macaques. The older, more dominant males often display maternal-like behavior. Gary Mitchell at the National Primate Center in Davis, California, found that young (subadult) males were more paternal than full adults. Paternal behavior may be characteristic of younger and very old dominant males.

Apropos of Dr. Williams-Ashman's earlier question about what happened to isolation-reared monkeys at puberty, Dr. Hamburg said he thought that insufficient attention had been paid to puberty and the following period in primate studies, especially in the wild chimpanzees. Although it has not been studied systematically in the isolation-reared animals, he thought there were indications that the males become much more aggressive, really prone to violence, after puberty.

In the wild chimpanzees, the following kind of behavior had been of great interest: around the time of puberty, the adolescent males take a great interest in the adult males, particularly in the highest-ranking ones. They will sit for long periods observing an adult male, gradually edging closer to him in an ambivalent way—three steps forward and two steps back—with indication of fear, but with a kind of absorbed interest that they have not shown prior to puberty. This interest reaches a point of climax in which the dominant male will beat the adolescent rather severely. Despite such beatings, the adolescent will come back in a few hours, go through the same sequence, and usually be beaten again. If that sort of thing goes on for several days, it appears to the human observer that the tension will become unbearable. But then he sees that the adolescent goes off into the forest alone. He will stay alone in the forest for a day or two and then rejoin the group.

The adolescent male for a couple of years following puberty displays an accentuation of some of the submissive behaviors. For example, there is a bowing pattern that all chimpanzees show, a pattern that becomes almost a caricature, a sort of bobbing. Dr. Hamburg and his son had seen it only in adolescent males toward the dominant adults. It is different from any behavior they show in any other context—a vividly submissive behavior.

Another aspect of the behavior of the postpubertal male, Dr. Hamburg continued, is that he is very rough if there is no dominant male around. He will severely attack females and young. There are certainly dramatic changes following puberty in the male chimpanzee. There are no corresponding changes in the females.

In monkey groups, Dr. Jensen said, the subdominant males learn their place and do not become overly aggressive when the group leader is present. However, they wait for their chance, and if the leader shows weakness or disappears, younger ones may take over. One of them will then often display leadership behavior, given the chance. That is probably one of the main ways that a change of group leadership comes about.

Dr. Price remarked that the speakers apparently considered androgenic hormones important in aggressiveness, to which Dr. Hamburg assented. Dr. Jensen added that studies of rhesus monkeys on Cayo Santiago Island in the Caribbean showed that some males who were castrated and then returned to the group could hold their rank for years, and even continued mating behavior at the same frequency as before. However, others were greatly affected. If they were castrated before puberty, effects were greater.

COGNITION: INTRODUCTION

John Anthony Ambrose
Center for Advanced Study in the Developmental Sciences, London

Consideration of what is actually meant by cognition might be helpful to begin with, suggested Dr. Ambrose. Most people think, with some justification, that the term has to do with intelligence and with learning. Indeed, the development of intelligence in the human being is very much tied up with cognitive functioning.

Today's psychologists no longer think of intelligence as something that is wholly genetically determined. The dichotomy between learning and maturation has now been eliminated sufficiently for us to understand that the development of intelligence has much to do, not just with learning, but with learning to learn. Basic to this process is learning to behave in ways that take account of things that are not in the here and now—people, objects, situations, and concepts that do not give rise to immediate sensory input because they are removed in time or space—things that are represented in the brain as signs or symbols.

The principal kind of symbol system we use, continued Dr. Ambrose, is language. Achievement of the use of language, in inductive and deductive thinking and in logical operations of all kinds, involves a highly complicated process of development. For psychologists interested in the development of human behavior, the study of cognitive development is an exceedingly important area. Until quite recently, the person who has done most in this area has been Jean Piaget of Geneva. His classical work, based chiefly on observation of his own children extending through many years, has laid the foundation for much research by others. Of the important new empirical work done in the last five to ten years, some has confirmed and some has extended Piaget's thinking.

Most people know that children learn some things much more rapidly at one age than at another. In particular, teachers know that if children do not learn certain things at a certain age, their cognitive development can be seriously impeded. Awareness of these facts has led psychologists to believe that the concept of critical periods, which originated in embryology, can be helpful in understanding some of their findings. Hence the relevance of the consideration of cognition to the general topic of this symposium.

A significant point to bear in mind now is that, in the development of human cognitive functioning, the earliest period of life, in which the human being is operating without language, is extremely important for understanding how language develops. Dr. Kagan will be talking about some research in the first two years of life that is relevant to our understanding of early cognitive development.

One of the exciting things about this and other recent research, Dr. Ambrose noted, is the increasing evidence of sexual differences in functions related to cognitive development—differences not so much in intelligence level as in the stability or consistency of the development of some of the primary functions. It is hoped that the discussion to follow Dr. Kagan's paper will bring out both the biological basis of cognitive development and the relation of that development to environmental influences.

A SEXUAL DIMORPHISM IN VOCAL BEHAVIOR IN INFANTS

Jerome Kagan
Harvard University

Psychology, sociology, and anthropology are sixteenth-century sciences, when compared to modern physics and chemistry, for the behavioral sciences do not have a metric and can make only the most general qualitative statements. The behavioral sciences require not criticism of their frail collection of theories and facts, but tolerance for a complicated and immature structure. One of the serious problems is the fact that lack of strong methods to study covert mental processes limits the inferences we can make about overt behavior. Since the same overt response can result from different covert processes (that is, one phenotype derived from different genotypes), it is necessary to find strategies of deciphering the varied meanings of significant classes of behavior. Comparative psychological research suggests not only that male and female animal forms behave differently in the same situation, but also that the same response may have different meanings in the sexes. There is every reason to believe, therefore, that there will be psychological or behavioral dimorphisms in the human being. Most of the sex differences in adults have been explained as a function of culture. Ten-year-old boys are more aggressive than ten-year-old girls; ten-year-old boys want to be scientists; ten-year-old girls are less so inclined; and the culture encourages these sex differences. There are over a thousand articles on sex differences in mental functioning in children and adults. Although different variables and different tests have been used, there are two replicable findings. The first is that females perform better than males do on any test of language skill. The girls have higher reading scores, better memory for language, and begin to talk earlier. The second reliable finding is that boys do better than girls on noverbal spatial tasks. The preferred interpretation of these two generalizations is that these differences are probably learned in the first three or four years of life. It is assumed that mothers talk more to their girls than to their boys and, hence, girls are good at language. Boys are encouraged to play with cars and mechanical objects and, therefore, it is concluded that they should be good at nonlanguage, mechanical problems. Reports from R. W. Sperry's laboratory (Sperry, R. W., 1964), however, should cause us to question the simplicity of this explanation. Performance profiles from adult patients who have had the corpus callosum cut for therapeutic reasons indicate that quality of performance on nonverbal spatial tests is better when the right hemisphere is doing the work; the quality of language performance is better when the left hemisphere is doing the work. This finding suggests there may be a biological basis for this sex difference in language versus nonlanguage competence.

This talk deals with sex differences in the significance of vocalization and verbalization during the first twenty-seven months of life in a sample of infants living in the Cambridge-Boston area. The data suggest a sexual dimorphism for this class of behavior. The question can be stated formally. Is it possible that the human male and female infant differ with respect to the prepotent response exhibited in a state of "attentional excitement"? We shall argue that there are differences between infant boys and girls in the tendency to vocalize when each is interested in an external visual or auditory stimulus. The data come from a sample of about 160 healthy, first-born, white infants. The children were seen in the laboratory at four, eight, thirteen, and twenty-seven moths of age and visited in the home when they were four and twenty-seven months old. *Method.* At four months of age the infant was placed supine in a crib and the stimuli presented above him. At eight and thirteen months he sat in a high chair with the mother sitting a little behind him and to his right. The visual stimuli were presented about twenty inches from the baby's face. The auditory stimuli were presented through a speaker baffle placed above and to the right of his head.

At four and eight months of age, the infants were shown stimuli representative of faces. (Fig. 1 shows these stimuli). Each stimulus was shown singly for a half-minute a total of sixteen times. Thus, each face appeared four times.

At all four ages—four, eight, thirteen, and twenty-seven months—the children were shown a set of clay masks painted flesh color. (See Fig. 2 for these stimuli.)

At thirteen and twenty-seven months of age the children were shown human forms—a regular form and six different transformations of that form. Finally, at eight and thirteen months the children heard a tape recording of a male voice speaking four different sets of sentences, each

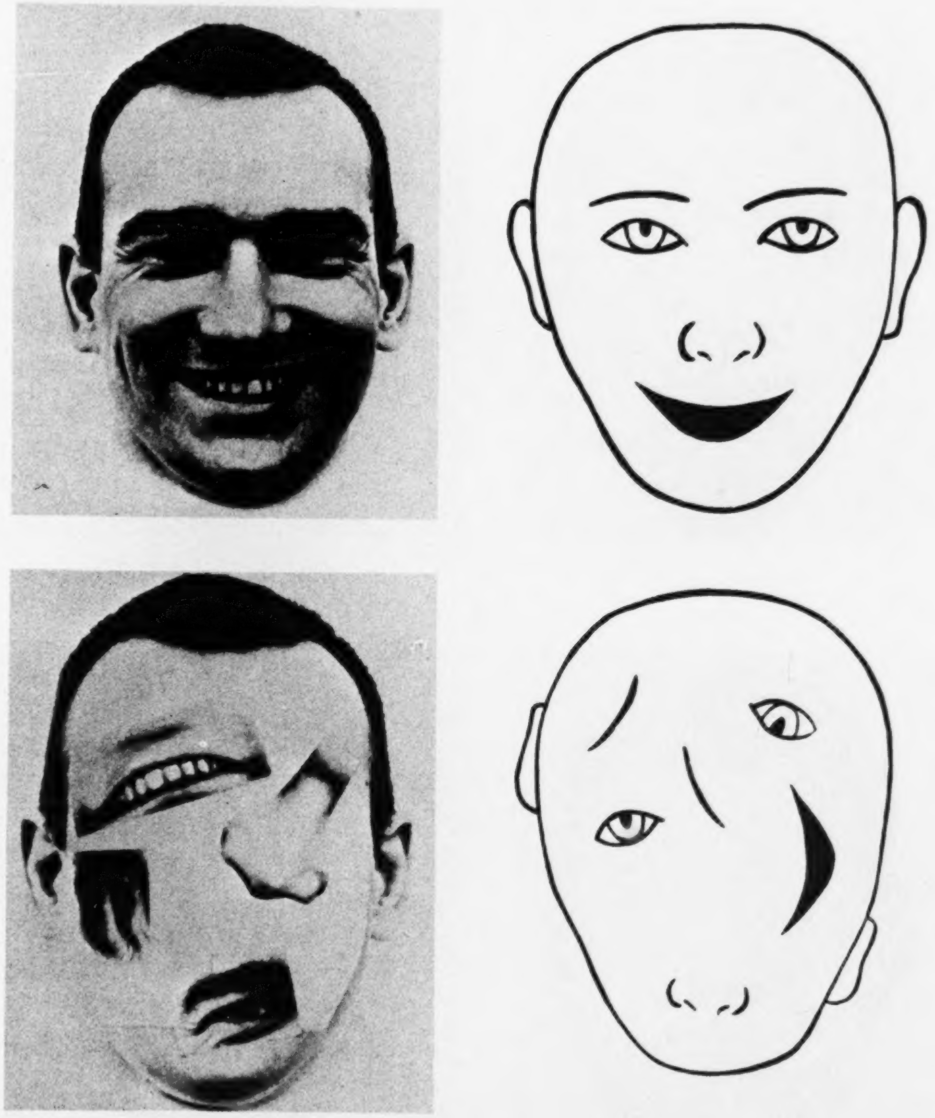

FIGURE 1.—Faces shown to infants at four and eight months.

twenty seconds in duration. Two of the stimuli contained meaningful words, but one was read with normal inflection and the second with no inflection. The other two stimuli were nonsense words, one read with normal inflection, the second with no inflection. Each of the four stimuli was presented three times.

The variables coded for the visual and auditory episodes were: the duration of the fixation of the visual stimulus or duration of orientation toward the speaker baffle, vocalization time, changes in heart rate, smiling, and fretting.

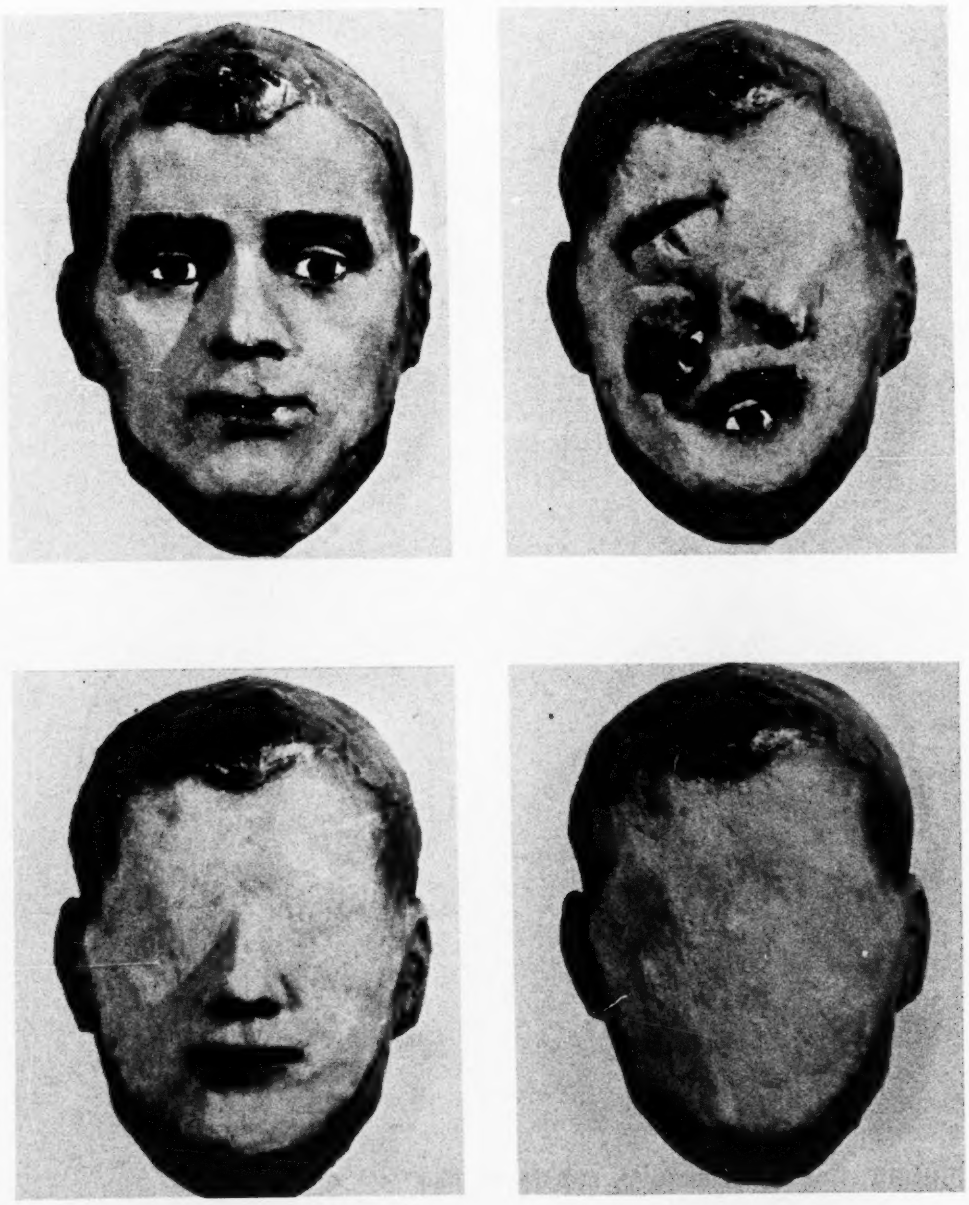

FIGURE 2.—Clay masks shown to infants at four, eight, thirteen, and twenty-seven months.

It should be noted that at four, eight, and thirteen months, the infants' vocalizations were nonmorphemic, usually about two seconds in duration. At twenty-seven months all their vocalizations were meaningful speech, including phrases like, "What's that, Mommy? What face is that?"

Cardiac deceleration was quantified because of the hypothesis that when the infant is attending to some event he is likely to display a decrease in heart rate. The preferred interpretation of this phenomenon is that it follows Obrist's suggestion (Obrist and Webb, 1967) that cardiac deceleration is accompanied by a decrease in motoricity. Since decreased motoricity characteristically accompanies attention, this is a reasonable hypothesis. The cardiac deceleration typically begins as the child fixates a stimulus and usually reaches its nadir within five seconds, often a little longer for an auditory event.

Results. The major results can be summarized succinctly:

1. The tendency to vocalize to the visual or auditory stimuli is more stable for girls than for boys across the first two years of life.
2. The tendency to vocalize is positively correlated with independent indices of attention for girls, but not for boys.
3. Increases in the tendency to vocalize across age were correlated with social class for girls, but not for boys.

Let us document these conclusions.

Stability. There was good intra-individual continuity for vocalization among the girls from eight to thirteen and from thirteen to twenty-seven months, but minimal stability for boys across these episodes. There were significant sex differences in the magnitudes of these stability coefficients. (See Table I for correlation coefficients.)

Vocalization and Indices of Attention. Vocalization following termination of the auditory stimuli at eight months was highly correlated with indices of attention. These children tended to show large decelerations during the presentation of the auditory episode. That is, the girls who vocalized when the voice terminated showed the largest cardiac decelerations during the presentations of the voice on the preceding trial. This did not occur among the boys. It appeared that the tendency to vocalize a few seconds after listening to a twenty-second speech sample was a relatively sensitive index of an attentive excitability dimension in the eight-month-old girl that was stable during the second half of the first year. This assumption is supported by analysis in which the girls with the ten highest vocalization scores to the auditory episode at both eight and thirteen months were compared with the ten girls who had the lowest vocalization scores at these two ages. Six of the ten high-vocalizing girls were upper-middle class, whereas only three of the low-vocalizing girls had college graduate mothers. Other data indicate that these girls had more finely articulated schema for faces and for their general life space than did the low-vocalizing girls.

The Relationship between Changes in Vocalization and Social Class. Although the absolute amount of vocalization at either eight or thirteen months was not related to social class, an increase in vocalization from eight to thirteen months did covary with the level of parental education. Girls who increased in vocalization were more likely to come from upper-

TABLE I—Stability of vocalization

		8 months				8 months			
		Three dim faces				*Auditory*			
		Tr 1		Tr 1-16		Tr 1		Tr 1-12	
13 months		B	G	B	G	B	G	B	G
HF I	Tr 1	17	11	28*	02	03	–01	14	–02
	Tr 1-12	03	11	39**	39**	00	28*	25*	34**
Three Dim Faces	Tr 1	–07	51**	–07	43**	06	45**	–02	27*
	Tr 1-15	–06	32*	–15	47**	03	29*	06	46**
HF II	Tr 1	–03	10	06	32*	18	30*	15	27*
	Tr 1-12	–02	06	00	26*	09	12	26*	28*
Auditory	Tr 1	–07	08	–02	28*	12	09	16	32*
	Tr 1-12	03	13	14	21	05	19	18	28*

* $p < .05$
** $p < .01$

	13 months				13 months			
	HF I				*Three dim faces*			
	Tr 1		Tr 1-12		Tr 1		Tr 1-15	
27 months	B	G	B	G	B	G	B	G
Slides:								
Verbal Discrepant	–07	10	–09	01	09	13	11	06
Verbal Nondiscrepant	–14	05	–19	06	12	16	16	15
HFI								
Verbalization	–15	28	–09	39*	13	43**	–20	37
Three Dim Faces								
Verbalization	21	40*	–19	47**	21	23	–01	07
Free Play								
Verbal Adaptation	–06	27*	13	30*	23	38**	31**	39**
1-3	01	26*	23	15	09	34*	26*	16
4-6	–03	32*	–01	34**	04	32*	14	17
Vocabulary Recognition	–26*	23	–14	–10	23	01	22	–18
Vocabulary Naming	12	16	–04	02	21	–09	15	–23

* $p < .05$
** $p < .01$

middle-class families. In the first analysis a ratio was computed to evaluate the proportion change in vocalization from the first episode at eight months to the first episode at thirteen months. The highest ratio possible was 2.0, a ratio of 0.0 signified no change, and a negative value signified a decrease in vocalization. There was an approximately monotonic relation between degree of increase in vocalization and parental education. The average ratios for four different educational levels were .07, .64, .60, and .96 ($p < .01$). Lower-middle-class girls showed no increase in vocalization, while the upper-middle-class showed a 50 percent increase. There was no such change for the boys. This increase in vocalization from eight to thirteen months among upper-middle-class girls should be correlated with parental encouragement and long periods of reciprocal communication between parent and infant. However, the mother is more likely to be responsive to a babbling infant than to a silent one; hence it is reasonable to expect an interaction between the young infant's tendency to vocalize and his experience at home. That is, we assume first that high vocalization scores at four months reflect the temperamental tendency to express excitement through vocalization and, second, that a babbling girl born to a better-educated parent will show larger increases in vocalization than one born to a less-well-educated parent. The data lends some support to the pair of ssumptions. We divided the distribution of four-month vocalization to the achromatic faces into thirds, with about twenty-four children in each group. We then compared children from better-educated versus less-well-educated parents within each vocalization tertile at eight, thirteen, and twenty-seven months. That is, we inquired of the future behavior of infants from families above or below the median on the educational variable, all of whom were in the bottom third of the vocalization distribution at four months, middle third of the vocalization distribution, or the top third of that distribution. There was generally a monotonic relationship, for girls only, between an initial tendency to vocalize at four months and vocalization to human forms at thirteen months and vocabulary score at twenty-seven months. This relation was better for middle- and upper-middle-class girls than for lower-class girls. To paraphrase, an upper-middle-class girl who is a frequent vocalizer at four months was more likely to be a high-vocalizing, high verbal girl at two years of age than was a high-vocalizing infant who came from parents who had not attended college. This relation did not emerge for boys. That is, social class had a more critical influence on verbalization and vocalization performance of girls than boys.

AN INTERPRETATION

This sexual dimorphism found in our longitudinal study has parallels in other investigations. For example, a study of six-month-old infants at the Fels Research Institute revealed that girls vocalized significantly more to faces than to geometric forms, whereas boys showed equivalent vocalization to both classes of stimuli (Lewis and Kagan, unpublished).

Zelazo (1969) found that three-month-old girls vocalized differentially to male and female experimenters looking down at their faces, while male infants showed no such differentiation.

The predicitive power of volcalization is supported by the research of

Cameron, Livson, and Bayley (1967), who reported that vocalization on the Bayley intelligence scale during the second half of the first year predicted Stanford-Binet IQ scores for girls during childhood and adulthood, but not for boys.

Finally, Moore (1967) reports that high vocalization scores on the Griffiths infant scale at six and eighteen months are stable for girls, but not for boys. Further, there is a correlation between the child's social class and the speech quotient at eighteen months for girls, but not for the boys. The entire corpus of evidence suggests that vocalization to representation of the human faces or speech reflect attentional processes with greater fidelity among girls than boys.

There are two possible interpretations of this replicable generalization. An environmental position argues that mothers who are motivated to accelerate their daughters' mental development will spend much time in face-to-face vocalization with them—more time than they would with a son and more time than mothers who are not concerned with their infants' rate of development. This maternal practice should lead to increased levels of babbling in the girl and articulated schema for human speech. This accelerating and concerned mother should continue to stimulate her daughter by teaching her words and encouraging the development of other cognitive skills. The predictive link between early babbling and quality of cognitive performance in the future will be a function of the continuity of the mother's acceleration of her daughter's development. The absence of this link in the boy requires the assumption that accelerating mothers do not preferentially engage in reciprocal vocalization with their sons. Preliminary data support this assumption, for observations of the mother-infant interaction in the home at four months of age reveal that well-educated mothers spend much more time in distinctive vocalization with their daughters than do poorly-educated mothers. There are no such class differences for sons. The best support for class differences in mother-daughter interaction comes from observations of thirty middle-class and thirty lower-class white mothers and their first-born ten-month-old daughters studied by Stephen Tolkin. Time-sampled observations of the mother-daughter interactions were made in the home on two occasions. The better-educated mothers engaged in significantly more face-to-face vocalization with their infants, as well as more reciprocal and distinctive vocalization than the poorly-educated mothers ($p < .001$ for most comparisons). However, there were no class differences in the amount of physical manipulation—holding or kissing. As might be expected, the middle-class children showed better signs of differentiation of speech when they came into the laboratory. Thus the sex differences reported here could be solely the result of differential interaction patterns with the parent, primarily the mother.

The second interpretation has a biological flavor and hypothesizes basic sex differences in neurological organization of the central nervous system mechanism serving vocalization and speech. Vocalization in the infant female may be a prepotent reaction to the arousal occasioned by the processing of interesting events. This speculative position finds support in varied classes of observations. Infant girls appear to be more aroused or affected by auditory stimulation than boys, for a soft 1000 cps tone is a reinforcement for ten- and fourteen-week-old girls, but not for boys (Watson, 1969).

Perhaps the typical dominance of the left cerebral hemisphere over the right emerges earlier ontogentically and is more clearcut in the female than in the male. If the hemispheres were less equipotential for females there might be a closer functional relation between information processing and expressive vocal sounds among girls than boys. This suggestion is supported by a series of independent studies conducted by Kimura (1967). When pairs of different digits are presented simultaneously through earphones, more digits are accurately reported for the right ear than for the left ear. The right ear advantage indicates that the left hemisphere is preferentially processing information. The dichotic digits task was administered to boys and girls five, six, seven, and eight years of age. Although both sexes showed left hemisphere dominance at the three older ages, at age five only the girls showed a significant superiority of right over left ear, while the boys' scores indicated less clear dominance. This left hemisphere advantage holds only for language and does not hold for nonlinguistic sounds, such as melodies or familiar environmental sounds. Further support for the notion that language competences are more elaborated in left hemisphere for females comes from a study in which right or left temporal cortex was removed from male and female adult epileptic patients who were tested one year after surgery on the adult Weschler-Bellevue Intelligence Test (Lansdell, 1968). Removal of left temporal cortex had a more serious detrimental effect on verbal functioning on female than on male patients. Removal of right cortex had a more detrimental effect on quality of functioning on nonverbal tasks for males than for females. The data suggest tentatively that the left hemisphere dominance accompanying language elaboration is less equivocal for females than for males. This suggestion is in accord with the finding that among dyslexic children, girls show the typical left hemisphere dominance in dichotic listening tasks, while dyslexic boys do not (Taylor, 1961). The data, therefore, are consistent and reliable and invite theoretical attention. The hardiness of the results makes it likely that we are dealing with an important psychological phenomenon. Comparative psychologists acknowledge that behavioral reactions to varied states of arousal are different between closely related strains or between sexes within strains. Vocalization in the infant female and male may be another such sexual dimorphism.

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DISCUSSION

Dr. Martini asked whether the deceleration of heart rate that Dr. Kagan had mentioned could be the result of increase of blood pressure and secretion of catecholamines, and whether this could be secondary to the signal stimuli. Dr. Kagan answered "yes" to both questions.

The explanation, Dr. Prechtl said, is in the breath-holding: there is an apnea and therefore bradycardia. What might be somewhat misleading is that the children become tense. With muscle activity, the heart rate goes up. In the case of the children, there is only a shift in body posture in the orienting response, especially to the sound stimulus, not an increase in muscle activity. Dr. Kagan added that sometimes the deceleration can be caused by a change in respiration, but not all the time. Both Lacey and Obrist have demonstrated that when the subject controls his breathing there can be deceleration. This work was done with adults, not infants, Dr. Prechtl noted. Dr. Kagan considered it reasonable to conclude that, if it were possible to get infants to control their breathing, the phenomenon would be the same as in adults. "Sometimes we find a respiratory change accompanies decelerations, sometimes we do not. Sometimes there is deceleration when the respiratory record has not changed at all."

To Dr. Caldeyro-Barcia's question as to whether there is any relation between the amplitude of the pre-existing variations in the heart rate and that response, Dr. Kagan replied that there was not anything consistent or dramatic.

Dr. Caudill emphasized what Dr. Kagan had said about the imitative relationship between the mother and little daughter, underscoring it from his observations on Japanese and American children at the age of two and a half years. "You have two women together when the child is at that age," he said. "The little girl is trying to vacuum the floor and wash dishes and do other things with her mother in a way that is much more likely to give rise to vocal interaction between them than would be true for the boy. This interaction starts very early in the mother's set toward the girl or boy."

"Where vocalization is the preferred mode of interaction, which is the case in my American data and not particularly so in the Japanese," Dr. Caudill continued, "then in the American data there is a significant correlation between the amount the mother chats to her infant and the amount of the infant's happy vocalization, but there is not this relationship in the Japanese data. If we analyze the data sequentially over time, we find that the American mother responds more quickly to her infant's vocalization. And she responds differentially to the unhappy and the happy ones, being faster to respond to the unhappy cries. The Japanese mother takes longer to respond to either kind of vocalization, and she does not discriminate between the two."

It is clear, Dr. Kagan interpolated, that environmental variables shape vocalization. The interesting question is why this is less true for boys. Is there any biological basis to this difference?

Dr. Caudill said that a preliminary analysis of his data showed that at two and a half years and again at six years in both cultures the girls vocalize more than the boys do. He intended to do final analyses on the data. He also had found that children who were low vocalizers at three to four months remain low vocalizers at two and a half and six years. There may be some interesting sexual differences involved here.

Cross-cultural comparisons are important, Dr. Caudill added. If one compares Howard Moss's American data with the American and Japanese data that Dr. Caudill had collected, one finds exactly the same thing: vocal interaction is an important matter in America and not so important in Japan.

Also, Dr. Caudill did not find sex differences in patterns at the age of three-to-four months, which is similar to Dr. Kagan's not finding sex differences at four months, though Dr. Kagan does find such differences by eight months. So one theoretical idea that emerges is that whatever happens to these children in the way of interaction between biological and environmental influences happens very early. Cross-culturally speaking, what gets built into the child first are the aspects of his culture by three-to-four months of age in Caudill's data, with the sex role secondarily on top of that by eight months of age in Dr. Kagan's data. Therefore, developmentally people may be thought of as creatures of their culture first, and, so far as environmental influences go, creatures of their sex second.

Dr. Kagan spoke of an observation made in Guatemala with the INCAP project under Dr. Canosa in connection with some experiments with four-month-old infants in a village called Akhatanondo. If you take an American four-month-old baby from his mother and put him in a crib, everything is fine. But the Guatemalan child of that age cries with fear. He does not want to be taken from his mother. He is used to being carried around. The cultural differences are apparent at that age. Dr. Kagan therefore agreed with Dr. Caudill that we have heretofore underestimated how early very important psychological patterns are established. The patterns are malleable, plastic, but the child is being shaped very early.

Dr. Caudill added that he did not know what particular value one should place on the ability to vocalize. Our culture places an enormous value on it. But children in Japan are brought up with the expectation of becoming Japanese. A highly verbal Japanese is not going to have as good a time in his own culture as will one who is more nearly normal. Hence Dr. Caudill did not know what is good or bad. To Dr. Kagan's remark that there is no good or bad, Dr. Caudill said, "There might be. The ability to manipulate language probably has certain values. And you did not distinguish between spontaneous or even elicited vocalization and reading. I find that at six years my American children vocalize more and better than do my Japanese children. But the Japanese children, possibly because of the nature of the language, find it easier to learn to read. They do much better at reading and writing than do the American children at that age."

Dr. Kagan said that he did not believe that infant babbling had anything to do with later reading ability or IQ. He and his associates are studying vocalization as one would study following in ducks, or grasping in rhesus monkeys.

The evidence so far produced for the biological basis of the sex differences seemed not really convincing to Dr. Lambo. He would prefer to see the simple experiments done in another culture that places a low value on vocalization, a culture in which there is no preferential treatment given to male or female children. He wished to know also whether there is consistent correlation within each social class. He was assuming that there would be minimal preferential difference in Dr. Kagan's working on lower-middle-class families as compared with the high social class. Dr. Kagan replied, "Not in America."

Dr. Tarkowski asked whether it might be illuminating to carry out studies like those Dr. Kagan had made with orphans—children brought up in conditions in which the attitudes of the adults who take care of them are more or less similar. Dr. Kagan remarked that if the implication was that the caretakers in orphanages react the same to boys and girls, it was a mistake. Dr. Tarkowski said he was not implying that, but that the attitudes would not be comparable to those of mothers and fathers at home. Dr. Kagan referred the question to Dr. Papousek, who had observed many children in the Institute for the Care of Mother and Child in Prague.

Dr. Papousek replied that his observations applied only to children up to six months of age. One must take into consideration, he said, that different interpretations are necessary of relations between caretakers and infants with respect to sex and also to other individual differences. So experiments would have to be controlled much more strictly. Dr. Kagan agreed, because the question is not a matter of obvious things, but of very subtle ones. Just looking at the infants, one would not see it at all.

The question of the right-handed or left-handed individual and the dominance of the left or right hemisphere interested Dr. Sundaram. Dr. Kagan said that from his reading of the literature on handedness he was persuaded that the difference is a poor index of whether the left hemisphere, the speech hemisphere, is dominant. He referred the question to Dr. Prechtl, who concurred.

Turning back to a point related to the questions raised by Dr. Lambo and Dr. Caudill, Dr. Kretchmer asked whether Dr. Kagan's statement that the language performance of girls is always better than that of boys is true cross-culturally. Dr. Kagan replied that it is in Vernon's studies in Africa and it is true in Europe, but of course that is Western culture. He asked Dr. Caudill if it is true in Japan. Dr. Caudill said that it is borne out by a preliminary examination of his data on the two-and-a-half-year and six-year-old children. Dr. Minturn added that it is true of American Negroes irrespective of social class.

With all agreed that girls use words earlier than boys do—and read better, Dr. Kagan noted—Dr. Garn asked whether there is not a difference at eight and twenty-seven months in Dr. Kagan's data about the amount of babbling that consists of words, as apart from the amount that is just coos and babbles. Dr. Kagan answered that there was, but that it is remarkably low. Girls are slightly higher. He had not mentioned that point because it is subtle. He was not sure of its statistical significance.

Dr. Minturn asked further about social class influence. If upper-middle-class mothers talk more to their girls than lower-middle-class mothers do, is there a difference between the amount of time that mothers talk to girls as opposed to boys? "Face-to-face, yes," Dr. Kagan answered. "Both Moss and we find that. But it must be face-to-face, not connected with any other activity, such as diapering." "Is there any evidence that lower-class mothers talk more to boys than to girls?" asked Dr. Minturn. Judging only on the basis of his own data, which are based on lower-middle-class families—blue collar workers' families—not the unemployed, Dr. Kagan said he found no difference on that point, but that he would want other samples. Dr. Minturn was correct, he said, in her understanding that upper-middle-class mothers talk to their girls face-to-face more than to their boys. As for the difference between mothers talking to boys and to girls in the lower-middle-class families, there is a slight difference favoring the girls, but it is much more striking in the college graduates.

In response to Dr. Lambo's question whether, in view of what Dr. Kagan had said about the importance of face-to-face talking, what he had said earlier about the lack of influence of television might indicate that a human agent must be involved, Dr. Kagan said that was not the case. Psychologists know that it is a varying set of graphs. Back in the 1930's, before television, Dorothea McCarthy made measurements of verbalization from boys and girls in lengths of sentence, length of utterance, and vocabulary and found that girls were considerably ahead of boys. In 1961, Mildred Templin studied children in Minneapolis, where the original study was done, and found that there are no longer any sex differences. On the basis of comparison of the means in 1931 and 1961, the boys have improved and the girls have stayed about the same. Her interpretation was that the boys have been watching television, which stimulated them (Templin, M. C., 1957). *Inst. Child Welfare Monogr.,* Ser. 26, Univesrity of Minnesota Press).

Dr. Lambo asked whether there was any emphasis in pattern, any additional factor to the individual actions of involvement in the way of contact. Dr. Kagan replied that both Moss and he had found that mothers do a good deal of tickling, holding the boys in the air, while they talk to them. But they thought that this fact has little to do with stimulating vocalization because the child's attention is devoted to the kinesthetic tactile stimulation.

Dr. Richardson suggested that there may be a difference between what a mother says to a boy and what she says to a girl in that with a boy she may be ordering him, telling him to stop doing something, etc. There may be a difference related to biological activity. This is true, Dr. Kagan noted, after the age of about eighteen months.

In response to Dr. Money's question whether he had given any thought to sex differential and development between boys and girls on this question, Dr. Kagan said he had. Many people had asked whether he would not have found a difference with the boys if he had studied them at six months instead of at four. His answer was that boys and girls are pretty close developmentally at the ages of four, eight, and thirteen months, though the patterns are different. If the studies had been made at four months and then again at thirty months, he would find this a legitimate criticism. Dr. Money said that needed some thought. There are so many ways in which boys differ from girls, starting with the birth rate, illness rate, dying-off rate. And girls are much more enticing to their parents in their responsiveness in the early ages.

A twenty percent displacement to correct for the appropriate developmental basis at the time does not change the nature or the direction of the data, Dr. Garn remarked. The intraboy correlations would still be zero, the intragirl correlations would still be .4. Dr. Kagan agreed. It might explain the fact that at eight months the boys perhaps were not cognitively ready to hear the auditory stimulus and, therefore, no relationship between the cardiac deceleration and the attention would be found.

CULTURAL DIFFERENCES IN PATTERNS OF SEXUAL BEHAVIOR: SEXUAL BEHAVIOR IN AFRICAN SOCIETIES

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Patterns of sexual behavior in cultures other than those of the West have never held much research attraction for behavioral scientists in general. There is a dearth of literature on the subject—chiefly observations made by missionaries, early anthropologists, and travelers, most of them biased and based on assessments made by tacit application of Western standards. I shall draw upon the findings of our work in Nigeria by sociocultural members of the society, buttressed by the work of others such as Tooth (1950), Carothers (1951), and Laubscher (1937) in other parts of Africa.

African societies present valuable material for social and psychological research because of their diverse cultures, with varying degrees of change from some that are highly sophisticated and westernized to others that are chiefly rural and still very traditional, having strong traditional social institutions, a number of which show great vitality and social value.

In Africa today, as in many developing areas, scarcely a year passes in which the social conditions of life—modes of conduct, habits, and amusements—are not subtly but surely altered. We have seen the shattering and in some cases ruthless annihilation of what we had considered the most stable, firmly established assumptions concerning African cultural institutions. Indeed, this very necessity for continual readjustment, the dynamic nature of the entire society, calls for intensive investigation of the interrelations between these broad social and cultural issues and the mental health of entire populations. With this unique opportunity, we have been able to study the general upheavals of sociocultural change and the tensions resulting from efforts to adapt to the process of modernization, to study the origin and development of normal and abnormal responses to new social demands, including sexual behavior.

TRADITIONAL AFRICAN FAMILY STRUCTURE AND SOCIAL RELATIONS

Since a meaningful comparison, in consideration of sexual behavior in the African context, must be made between the traditional, the changing (transitional), and the westernized groups of African population rather than by application of criteria based on socioeconomic status, such as education, occupation, and other parameters difficult to assess with confidence in these populations, it seems appropriate here to describe briefly the traditional social institution of the family, especially in relation to the sexual behavior of individuals.

Our recent review of child-rearing practices and personality characteristics in major African cultures, combined with clinical data systematically collected from psychiatric practice, as well as data obtained from interviews of a small sample of urbanized families, has led us to formulate a number of hypotheses. We have seen that it is not possible to make any but the broadest generalizations concerning these transitional African cultures. We have found that in the few cultures we have examined, the general characteristics of personality structure show that it is tradition-oriented, and from social-anthropological accounts of many other African cultures and societies, one can safely conclude that this is the general rule.

As in many other African societies where traditional culture still flourishes in considerable strength, in Nigeria the polygamous family, which is patriarchal and authoritarian, remains strong. The family system is extremely important in the social structure of African cultures. The traditional family is a very large one, consisting of a husband, several wives, children, the grandparents, uncles, aunts, and many cousins who may or may not live together. It is against this background of a closely-knit society with a well-defined value system, social roles and obligations, mores, and sanctions that we can understand the sexual behavior of individuals.

In 1959, when writing on the basic psychological structure of African populations, I observed that on the whole childhood is a happy period of emotional closeness between the young and the multiple members of the family. I commented on the general freedom and absence of threatening parental attitudes and on the exceptional kindness, affection, and consideration with which the family treats its young and growing members as part of their traditional education. The degree of freedom allowed is culturally conditioned. Social pressures, such as rigid rules about social relations, observance of religious and cultural rites, all lead to social conformity. Our observation has shown that parent-child relations in most, if not all, traditional cultures are direct, comprehensive, intimate, and less demanding than in Western cultures.

Because of the lack of inhibition, especially with regard to sex, children talk and observe adults freely. At a very early age, Nigerian children gain firsthand observational knowledge of the sexual act, and they imitate the adult sexual act among themselves without feelings of guilt. Sex education is completely informal. Children listen to adults' conversation on the subject. In many African cultures social premium is placed on male potency and masculine features.

SEXUAL BEHAVIOR WITHIN THE TRADITIONAL CULTURE

As an illustration of sexual behavior within a traditional culture, I shall discuss the pattern presented by the Yoruba tribe of Nigeria, which has been under intensive sociopsychological and anthropoligcal study for many years, with references to neighboring African cultures. The people in this group are very traditional, retaining the family pattern common to most traditional African structures as outlined above. They live in rural areas, and their villages are undergoing varying degrees of socioeconomic change.

In this and related cultures, the end result of all marriage is procreation. Yoruba families take pride in the large number of children in the household, even when family resources are precarious. The whole dynamics of sexual relationship vary considerably from what obtains among the urbanized and highly sophisticated Africans. Marriage is less the result of mutual attraction than of a "social contract" between the two families. During the education of young men and young women in this traditional society, special emphasis is laid on sexual relations. Adolescents from this environment are on the whole much better adjusted than are those in other cultures; they show ready acceptance of the social and biological roles required of their sex.

In our study of the psychological pattern of growth, we evaluated the physical changes that take place during this period (alteration in body proportions, changes in primary and secondary sexual characteristics ending in sexual maturity in boys and menarche in girls). Because there has been some cultural provision and preparation for these changes, the clumsiness, shyness, and awkwardness so often noticed in urban children of westernized homes in Nigeria are conspicuously absent in the traditional setting. The well-advanced and mature psychologic structure and social behavior support the manifold physiologic changes. Anxieties created by the turmoil of puberty and adolescence are frequently absent.

Extreme consciousness of the body has never been culturally encouraged in this culture. In fact, in the traditional African cultures children are often allowed to indulge in sexual desire without criticism. Their sexual organs may be played with or their sexual potentialities discussed at length and in detail before them by older persons. At a very early age they gain firsthand observational knowledge of the sexual act, and they imitate adult sexual activities among themselves, publicly when they are very young and somewhat more privately when they become older and more self-conscious.

Traditional cultures in Africa have also provided ways of stimulating the sexual organs. For example, in Dahomey, on the West Coast of Africa, "Girls who are from nine to eleven years old—that is, whose breasts are beginning to develop—are assembled by compounds in groups . . . and engage in the practice of . . . massaging and enlarging the lips of the vagina. They gather . . . at sundown behind the house of the woman in whose care they have been placed. . . . With a shaped piece of wood, this woman manipulates the lips of the vagina of each girl, pulling at them and lightly puncturing the vaginal tissue in several places. This she does eight to nine times for each of her charges during the first year of instruction, and during the next year the girls do this for each other . . . for two years at the very least this is continued, and in addition there is the outer massaging of these 'lips' to cause thickening and muscular development, for 'thin-lipped' women are considered lacking in comeliness." (Bettelheim, 1955)

Among the Baganda and the Suaheli, the girl before reaching puberty is encouraged to enlarge her labia through frequent pulling and stroking and by use of some special herbs or leaves. The psychologic meaning of the whole operation seems to derive from the expectation of its results and from the actual results. For example, sexual maturation (using crude criteria-stages of development of genitals, development of breasts, etc.) seems to be accelerated by these practices.

Though they have no direct relation to *rites de passage,* such practices, prescribed by the culture for boys and girls, obviously increase the desire and opportunity for masturbation and, according to the teaching connected with them, enhance sex enjoyment, promote sexual maturity, give positive motivation, and remove any form of psychologic guilt.

The atmosphere within and without the home, which is prescribed and emphasized by the cultural institutions, helps the youth (prepubertal, pubertal, and adolescent) to express his fantasies and desires freely and fully. This makes it easy for him to convey his feelings to adults, which he might not do in a more restrictive and inhibited setting.

In addition to the foregoing data on the methods prescribed by the culture, we have some findings on prepubertal sexual maturity from our studies of rural individuals and families.

PREMARITAL SEXUALITY

Of the 215 (70 husbands and 145 wives) married families interviewed in twelve traditional villages in 1958, only 23 men had not had premarital intercourse with their wives. On the whole, there is in this culture a now-disappearing practice that some intercourse with girls before they join their husbands is desirable. Nevertheless, the social pressures are strict and severe; these people are known for the violent attitudes they have toward sexual matters and for their strict sexual mores. Of this group of 215 married couples, there were two husbands who hesitated and thought that their daughters should not allow it until they received approval from them. Old and young men alike felt that the practice was essential, having been intended originally to enable the proposed wife to give proof of her fertility.

Premarital sexuality has been abused outside the traditional setting, especially by town-dwellers, migrant workers, and others who escape either temporarily or permanently from the social pressures and traditions of indigenous societies. Premarital sexuality is not uncommon among urbanized and educated Africans. The need to have children is paramount in the minds of every African—traditional, transitional, or westernized—and this influences to a considerable extent his attitudes to sexual relations. Kenyatta (1961), writing on the Kikuyu child, observes: "First and foremost he is several people's relative and several people's contemporary . . ." This makes illegitimacy almost an unknown status in Africa; children who are born out of marriage still have social recognition. Laubscher (1937) observes that they belong "to some male, either the man who procreated them or the mother's father . . ."

MARITAL SEXUAL BEHAVIOR IN THE CULTURES

The most significant finding in an intensive study of this sample was the total absence of any negative feelings or attitudes toward sex, such as one may encounter in other cultures, especially in our own culture organized on westernized conventions. Sexual relations were thought of by the majority of the women (142) as one of the most important ways of maintaining positive individual relationship with their husbands, although the pattern seems to be entirely "mechanical" by comparison with the Western cultures, in that each wife would take her turn with the husband as soon as she finished her menstrual period. Eighty percent (116) described orgasm during intercourse and had never experienced any form of negative feeling, frigidity, sexual aversion, or withdrawal of sexual interest; the remaining 29 did not want to discuss the matter in such detail, and 23 of this group of 29 wives had been married within one or two years before the interview took place.

The husbands described in great detail the warmth, physical pleasure, and other "positive feelings" inherent in sexual intercourse and thought that, although the main purpose was to procreate, sexual relations tend to draw husbands and wife or wives together and make their lives much more meaningful within the home. We noticed that the frequency of sexual intercourse varied considerably, but that on the whole the frequency was much higher (2:1) than among those in urban areas. In rural traditional communities the pattern of sexual behavior is, however, formal, less elaborate, rather routine. Women are never caressed, "played with" before coitus.

Older men in the village continue to test their virility and potency by marrying younger women. Of 44 men over the age of fifty years interviewed, all, without exception, showed continued interest in sexual life, spent a lot of money on "medicines" for "power." The older a man gets in this culture, the more anxious he becomes regarding his sexual power, which is equated with his entire life, his vitality, and his ability to retain his social power and position within the group. On the other hand, the women show decreasing interest as they grow older. In spite of the distinctive roles between men and women and the position of dominance of the men, sexual relationship is close and forms a part of the mutual obligation to the entire family and tribe as well as a biological expression of their feelings. According to Jomo Kenyatta (1961), "If a man dies without a male child, his family group comes to an end. This is one thing that the Kikuyu people fear dreadfully, and it can be said to be one of the factors behind the polygamous system of marriage."

It is, therefore, important to place this relationship among the total psychological functions attached to sex within the traditional African culture. It is part of the total emotional relationship within the family. Since earlier investigators inferred some biased and crude conclusions from their data, it seems relevant to refer to some of these observations and the consequent severe judgments on the sexual behavior of Africans.

Ritchie, in a study of the South African Bantu tribe, observed: "Many pass all the way from the last stage of infancy, through childhood and youth, right into maturity without ever having experienced voluntary sexual abstinence." An observation commonly made by many European research workers in the field is that the African, after what would seem to be a promising childhood with good foundation for balanced personality and emotional security, becomes hopelessly inadequate at, and after, puberty, and that this is due to his total preoccupation thenceforth in the pleasures of sex.

Perhaps what is most significant here in the study of the traditional culture, when compared with Africans who have been exposed to Western influences, who are urbanized and educated and have accepted a new style of life, is the total absence of any form of sexual perversions, abnormalities, and sexual crimes. An extensive study of sixteen traditional Yoruba villages as a follow-up of our previous study (Leighton, Lambo, and others, 1963) did not reveal any occurrence of sexual crime, abnormal sexual relationship, or even sexual promiscuity in any of these communities. In 1965, as a result of earlier work, I observed:

a. Indecent exposure and gross indecency: The consensus was that this has never constituted an offensive act, since most children in the tribal areas wear nothing until they are approaching puberty and young boys and girls as well as adults and elders wear the barest minimum. Children and adults of both sexes bathe together in village streams. The body-image concept has never appeared strongly in the psychology of the African—consequently such conditions as transvestism, exhibitionism, etc.. are for all practical purposes absent from the symptom structure of the rural African psychiatric disorder. The same sociocultural factors underlie the absence of "indecent curiosity" or "peeping Tomism" as known in Western culture as offenses in the traditional cultures.

b. Indecent assault, rape, assault and wounding: According to the elders, these acts or disordered behavior had not been recognized in rural and traditional communities except in the mentally ill, and even then sexual perversions or sexual crimes as symptoms of gross psychiatric disorder were conspicuously absent in the rural and traditional African (Lambo, 1965).

In contrast, Africans undergoing severe transitional stress in periurban or urban settlements manifest such conditions a prostitution, homosexuality, bestiality, and other abnormal modes of sexual expression. This is particularly marked in migrant laborers who are separated from their families. Individual cases have been studied, as well as examples of other criminal behavior associated with severe acculturation, social displacement, and isolation, giving rise to what we term "malignant anxiety" in culturally isolated, drifting Africans in urban areas (Lambo, 1962).

PATTERNS OF SEXUAL BEHAVIOR IN TRANSITIONAL COMMUNITIES

Transitional communities in Africa are those made up of individuals and groups that are undergoing rapid sociocultural change. The groups are mobile and hold a social position somewhere between the traditional rural and urbanized, westernized groups. In these groups are low-income workers, migrant laborers, and others working or searching for work in the urban centers, all of whom have moved from the rural areas.

The overt psychological reactions of the people in the transitional communities would constitute an interesting chapter of our study. In our 1957 "Survey of Displaced and Detribalized People in Yoruba Country" (unpublished), we found that the morbidity rate, as measured by crime, incidence of venereal disease, indiscriminate and prolonged use of marijuana, alcoholism, psychoneurotic and psychosomatic symptoms, was highest among those who were perpetually out of work, who did not have any member of their family with them, and who could be strictly termed "migrant laborers" of long duration. We concluded from these studies that migration—spontaneous or controlled—has much bearing on the adjustment processes of persons passing from indigenous to industrial economies, from one social class to another, or from rural to urbanized communities. We are confident that the correlation found between certain social factors and the high morbidity rate in this "captive population" is not due to chance, but reflects a trend in a population made up of individuals who are forced to maintain a marginal social relationship to society and are generally undergoing stress.

In our earlier study of socioeconomic changes in Africa and their implications for mental health, we observed over a two-year period that sexual offenses against women of a migrant group of workers were 472, of whom 123 (26 percent) had a definite history of taking Indian hemp (*cannabis*) and had only marginal occupation. Two hundred seventy-two (57.4 percent) had no fixed abode and were illiterate. Their ages ranged between 27 and 35 years.

Normal Sexual Relations

The same degree of insecurity, anxiety, and emotional difficulty that beset the lives of these people also characterizes their normal sexual relations. Out of 250 workers (mostly laborers) interviewed recently, 78 of

114 men who had migrated from other areas did not have their wives with them; 11 of them had lost touch, while the others (67) had made only periodic contacts with their wives, usually with unsatisfactory sexual relations. Most of them were suspicious of their wives' fidelity, and 62 out of the 67 who had infrequent contacts with their wives no longer experienced any deep feeling, physical or emotional, in their sexual relations. As a result of this and other factors, they took on "local wives," and as many as 23 had and continued to have sexual relations with local prostitutes; 5 had abnormal sexual relations entirely (homosexuality).

These men on the whole had profoundly altered their attitudes toward sexual relations, treating such relations in the same manner and deriving from them the same kind of physical pleasure as one would derive from alcohol or drugs (*cannabis India*). The values placed on, or traditionally assigned to, sexual relations in the culture had undergone marked changes. In some industrial centers in Africa, some workers are not allowed to bring their wives along with them while they stay in a "housing estate" (usually a large dormitory) built for them. Homosexuality has broken out on a big scale on many occasions, but the most disturbing aspect is the inability of these men to enjoy normal heterosexual relations with their wives again.

It is not known whether as a result of this situation the wives have experienced some significant changes in their attitudes, feelings, and behavior toward normal sexual relations. We did not have the opportunity to interview their wives, but we hope to do so.

The sexual behavior of westernized, urbanized Africans does not vary significantly from the behavior and relations often described in Western societies. But the most significant features, especially in contrast to those of traditional rural families, are the alterations in their attitudes toward sexual relations, the appearance of such conditions as sexual aversion and frigidity in westernized African wives, usually symptomatic of disturbances in their total relationship with their husbands. This is much more prominent among Nigerian professional women, e.g., nurses, teachers, secretaries, who have some form of emotional investment and commitment outside their homes.

These data have been taken from case histories of women who have consulted physicians for many medical conditions other than psychological, and therefore one should be very cautious in generalizing about them. There is no doubt that work of a more refined and well-planned nature remains to be done among the highly westernized Africans whose social and cultural conditions approximate those in similar sociocultural situations in many societies.

CONCLUSION

The data I have pulled together here are entirely fragmentary, but nevertheless emphasize that social, cultural, and environmental circumstances have definite effect on human behavior, including sexual behavior and responses in such a way that some times it may be hard to differentiate social inheritance from Mendelian law. I have used the cultural differences among three contrasting communities in Nigeria while the genetic variable is presumably held constant in the three groups, which are ethnically homogeneous, instead of looking more intently into such narrow criteria as religious, social, and occupational variables. I have pointed out these defects in my paper in order to stress the need for better and more precise observational work that remains to be done in collaboration with other disciplines.

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DISCUSSION

Transition from traditional to westernized cultures is a matter of worldwide importance, not at all limited to Africa, with distinctive feature and common themes everywhere, observed Dr. Hamburg. It is curious that so far economists are the only behavioral scientists who have written extensively on the subject, and their models of man are rather simple.

As Dr. Lambo pointed out, the transition produces stresses so great that they involve grave individual and social risks. We know little about how individuals in small groups come to terms with these stresses, what their coping behaviors are. As traditional guidelines to behavior are broken down, it is not clear what guidelines will replace them. This is a serious problem for behavioral scientists. Anthropologists have been more interested in it than have psychiatrists and psychologists.

Dr. Lambo touched upon a fundamental point, Dr. Hamburg continued, when he emphasized the importance of observational learning in a social context. With respect to sexual behavior, the sequence of observation, imitation, practice rests upon the continuing direct access of the young organism to the behavior of older organisms. Dr. Hamburg considered this perhaps the most important mode of learning in human evolution, not only in relation to sexual behavior, but also to aggressive and other kinds of behavior as well.

Dr. Feldman suggested that the changes in patterns of behavior and values described by Dr. Lambo as occurring with the movement from rural to urban societies might be explained by two kinds of mechanism: 1) that within the urban societies there is an artificial transplantation of values from the West, or 2) that some stresses inherent in the new social structures elicit change. He asked Dr. Lambo how much the changes he had talked about are related to the first kind of factors and how much they are related to the second.

Dr. Lambo replied that he had avoided using a term that Dr. Ribeiro would be using later in his paper: syncretism between cultures. "For example," he said, "if you take the area of religious conflict in many African cultures where this trait has been imposed on the indigenous cult system, you will find that in some areas where there is give and take on both sides there is a sort of syncretism, an ability to integrate adequately. This has occurred in Cuba and in Brazil, where the indigenous cult brought from Africa has been syncretized into the Roman Catholic faith, with adoption of the names of saints, etc."

In other parts of Africa where one is holding onto a major area, the conflict is much more concentrated. If people who come from the traditional, rural areas are able to form stable relationships and to maintain some social values in the urban area, they are able to make the transition as they go along. It is complete severance from the traditional that causes the difficulty.

Although there are only a few Catholics in Nigeria, Dr. Ribeiro asked Dr. Lambo whether he had observed that their effect on the pattern of the traditional family had brought any increase in sexual immorality, chiefly in relation to normal sex behavior. This question, Dr. Ribeiro said, is related to theories advanced partly to explain the homosexuality of the Negro. Some people suggest that the mother center, because of the common law relationship often found among American Negroes, would account for the mother-centered families with absent fathers and deserted women and a consequent increase in homosexuality.

Another question Dr. Ribeiro wished clarified, because it related to research on homosexuality he hoped to carry out in Angola, had to do with the practice, said to be common in Angola, of not only massaging the breasts of girls but of extending the clitoris to a very large size. Because of what had been said about the virtual absence of homosexuality in Nigeria, he was interested in knowing whether Nigerians practiced the enlarging of the clitoris and whether the practice had a bearing on anything else.

Dr. Lambo replied that the practice was not prevalent in Nigeria, but was in some neighboring areas, notably those he had mentioned. In studies of Nigerian society from different points of view he and his colleagues had discovered the existence of cults that tend to be sort of cultural trials for homosexual feelings. For example, there are two in Nigeria in which, twice a year, a man can dislike women and have breasts and not dress well. Obviously men who feel this way in Western cultures, in London and New York, will buy brassieres and show their symptoms in this way. But in areas like Nigeria or other parts of Africa there is a culturally conditioned or sanctioned channel for this practice, which is almost venerated. It may be that there is a genetic constitution that makes for homosexual feelings or latency in practically every culture of society. But a society that deprives people of a way of showing the feelings or latency in a culturally sanctioned way may force them to show the condition as symptoms. This, he added, is entirely a speculation.

VARIATIONS IN SEXUAL BEHAVIOR AMONG SOCIAL CLASSES IN THE U.S.

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For a long time, the more fortunate members of society in the United States have believed that the poor, with all their deprivations and frustrations, have enjoyed the pleasures of sex with greater freedom than have those in the higher socioeconomic classes. Whatever the hard knocks they could not avoid, at least for them sex was free and available. But recent sociological and anthropological studies made in Mexico, Puerto Rico, Jamaica, England, and the United States suggest that this view is not realistic. It seems more likely that the pains and privations afflicting disadvantaged people affect sexual behavior as much as they do behavior of other kinds, making it hard for the poor to develop a secure and gratifying sexual life.

"Lower class" as contrasted with "middle class" in the United States context is here taken to refer to the estimated 20 percent of American families classified as poor or near poor. The breadwinners in poor families have unskilled, highly unstable jobs; educational levels are low; the families live in the less desirable parts of cities and towns or in rural slums or migrant labor camps. Above them on the socioeconomic scale are the stable working class composed of people who hold semi-skilled manual jobs—the gray-collar workers in industry or in services, including policemen, firemen, and so forth with their families. Then there is the middle class, the white-collar class, including highly-skilled workers and technicians of various kinds.

For most men and women at all class levels, marital sexual relations provide the chief source of sexual expression. Other sources of sexual gratification are regarded as compensations or substitutes for normal married sex life. A study of sexual behavior can begin, then, with a comparison of the attitudes toward and evaluation of sexual relations by husbands and wives of the lower, working, and middle classes and the gratifications and dissatisfactions they find in them.

When social scientists interview men and women in detail about their sexual relations, there emerges almost immediately a continuum of interest and enjoyment ranging from great interest and enjoyment to strong rejection. The range is more apparent in women than in men. Men rarely express indifference to sex. Women present a gamut of responses from "If God made anything better, he kept it to himself," to "I would be happy if I never had to do that again; it's disgusting."

In one study I was involved with, we interviewed more than 250 middle-, working-, and lower-class couples and found that is was possible to categorize the responses of husbands and wives as showing great or mild interest in and enjoyment of sexual relations or slightly negative feelings, or outright and strong rejection (Rainwater, 1964).

In our interviews of middle-class husbands, there were almost no cases of negative feelings. Positive interest and enjoyment were overwhelmingly reported (something like 75%). The strong-interest responses decreased somewhat in the interviews with husbands of the working class and dropped off sharply with those in the lower class.

Among the wives, positive interest was expressed in the proportions of 50%, 53%, and 20% in the middle, working, and lower classes; mild interest ranged from fairly high (36%) in the middle-class group to very low (16%) in the working class. Negative feeling were expressed in the percentages of 14, 31, and 54 from the middle to the lower class. As with the husbands, the differences between the middle and working classes were not great, but they dropped off considerably between the working and lower classes.

Social class, of course, is only an indication of people's position in a social structure; it does not directly cause anything. Therefore, class differences are helpful only in showing the investigator where to look to find out what is making the differences. Having discovered such differences, one asks what accounts for them.

From other research, we have learned that the most important factor apparently bearing upon class differences in other areas of family relations has to do with the way the marital-role relationship is organized in different social classes. Middle-class couples, we have found, tend to emphasize a pattern of joint activities both in the home and in recreation outside the home. Whether husbands and wives actually participate together in such activities, each is expected to be interested in what the other does. In working- and lower-class families, much more frequent is a pattern of marital relations in which there is greater separation of husbands' and wives' activities. Particularly in the lower class there is a tendency for husbands and wives to go their own ways, with sharp divisions of labor and separation between the leisure-time activities of both.

What one needs to find out, then, is whether conjugal-role segregation accounts for these kinds of differences in sexual attitudes. We discovered that this factor does not account for the differences among couples in the middle class, but that in the lower class it accounts for a good deal of it. If we take a simple dichotomy of low or high segregation in the marital relationship, for the husband we find strong and mild interest in sex in the percentages of 72 and 28 for the less-segregated couples and in the percentages of 55 and 45 for couples with high segregation, with the wives showing mild interest and negative feelings in the percentages of 43 and 32 for the less-segregated couples and 14 and 66 for the more-segregated couples.

The figures show that, for the segment of working-class couples with lower segregation of marital role, there is no difference between them and the middle-class couples. In other words, the lower value placed on sexual relations by lower-class wives, and to a lesser extent by husbands in this class, can be seen as an extension of the higher degree of separateness in their general marital roles. Since the wife's interest in sex in Western cultures tends to be heavily dependent upon a sense of interpersonal closeness to, and gratification in her total relationship with, her husband, it is hard for her to find gratification in sex in the context of a high degree of separateness in the general marital relationship. When husband and wife are not accustomed to relating intimately to each other, it is difficult for them to achieve close and satisfactory sexual relationships. It may well be that a close sexual relationship has no particular social function in such a system, since the activities of husband and wife are organized on a separatist basis, and no great contribution is made by a sexual relationship in which they might sharpen their ability for co-operation and mutual relation.

Examination of the few cases in which, despite high separation, the wife enjoys sex indicates that this comes about when the wife is able to bring her own highly autonomous interest in sex to the relationship. To the extent that she is dependent upon her husband, as generally the Western tradition suggests she should be, for stimulation, encouragement, and understanding, on the other hand she seems to find frustration in sexual relationships.

This same general differing orientation to sexual relations is also apparent when one looks at what husbands and wives say about why they engage in sexual relationships, about what sex does for them, why it is important to them, why it is unimportant to them, and so on. Generally people outline two broad kinds of functions. One might be called psychophysiological relief. It gets rid of tensions, it relaxes, it gives physical relief. One man said it is like the back-pressure in a car that you have to get rid of. And it provides the sensual pleasure of orgasm. The other theme emphasizes instead the social-emotional gratifications that come from closeness with the partner, a growth of love, a sense of oneness, of sharing, of giving and receiving. When people talk about sex in this way, obviously they have in mind something that is different from psychophysiological relief.

Almost all men and women mentioned the physical aspects of sexual relations; there is no differentiation among couples on that score. But there is quite a bit of variation in the extent to which they mention the factor of social-emotional closeness. Here one finds that the differences in overall interest and gratification by class exactly parallel the other differences noted—i.e., middle-class couples and those with less segregated role relationships emphasize social-emotional closeness; lower-class couples and those with higly segregated role relationships emphasize the psychophysiological relief. This is true of both men and women.

In the area of nonmarital sexual relations, I can talk only about lower-class behavior, because, aside from the Kinsey studies, less research has been done on middle- and working-class behavior. My own research has been almost exclusively with a Negro lower-class housing project in St. Louis.

In general, from the Kinsey studies we know that there are fairly marked class differences by social status. Kinsey's main index of social status is the educational level. It seems clear that before the age of twenty, both boys and girls of the lower class are much more likely to have premarital coitus than are middle-class boys and girls. However, even lower-class girls are not as likely to have premarital sexual relations as are middle-class boys. The over-all double standard seems to operate at all class levels.

After the age of twenty for whites, status seems to influence premarital coitus in opposite ways for men and women. After that age, middle-class girls are more likely than are lower-class girls to have premarital relations, perhaps because the lower-class girls are already married, while lower-class boys continue more frequently to have premarital relations than do middle-class boys.

From the Kinsey studies, we know that there are great differences between white and Negro females in the extent to which they engage in premarital coitus. While the social class influence is the same in both groups in the teens, the level of exposure to sexual relations is on the order of three to four times higher for Negro girls than for white girls.

Thus, at the age of twenty, only 26% of white girls who left school after grammar school have had premarital sexual relations, whereas over 80% of comparable Negro girls have (Gebhard, P. H., *et al,* 1968).

White slum groups, according to anthropological studies done some time ago in Boston and more recently in Chicago, tend to grade girls rather finely according to the degree of their promiscuity, with virgins being highly valued and often protected. (This is a matter of the structure of the communities in which the white and Negro girls are growing up and the degree to which these communities attempt to control the sexual behavior of the girls.) One-man girls are still able to win some respect from those around them; promiscuous girls are quickly put into the category of "an easy lay."

In such groups, then, although boys are constantly exposed to stimulation to engage in sexual relations, efforts are made to protect girls, even to conceal from them elementary facts about sex and their future sexual role. Lower-class young women uniformly complain that their mothers first spoke to them about sexual relations the night before their wedding, and then tried to cram into one twenty-minute conversation all of the socialization that they felt was appropriate.

In the Negro lower class, the clear-cut difference between the amount of sexual activity permitted girls and boys, men and women, that seems to exist in the white lower class is absent. Instead, at the age of fifteen, according to the Kinsey reports, more grammar-school-educated Negro girls than white boys have experienced coitus. This is also true of the high school level. With over 60% of grammar-school-educated Negro girls having had intercourse by the age of fifteen and over 80% by the age of twenty, it seems clear that within the Negro slum community, whatever the attitudes involved, lower-class Negro girls are introduced to sexual relations early and, compared to white girls, engage much more frequently in sexual relations once they have started.

Boys employ well-established patterns of seduction within the Negro slum community. They are sharply judged by other boys and girls on their ability to employ these techniques. Boys show a good deal of anxiety lest they be rated low on these skills.

As is well known, this high degree of sexual activity leads to a high rate of illegitimacy, although there are other factors involved, too.

One might say, at least for this group, that the middle-class stereotypes of lower-class people as free to enjoy sex—the poor man's pleasure—is accurate, because here, after all, the behavioral data indicate a high degree of sexual participation starting quite early in adolescense, and at a play level even before that. However, close observation of ghetto peer activities of late adolescent and early adult Negro males and females indicates that this is not the case.

In the first place, attitudes toward sexual relations are highly competitive among one's own sex peers and heavily exploitative toward the opposite sex. Negro girls who do engage in sexual relations in response to the strong lines of the boys who "rap" to them, as the expression goes, often do not seem to find any particular gratification in sexual relations, but rather engage in sex as a test and symbol of their maturity and their ability to be in the swim.

Over time, a certain portion of these girls do develop their own appreciation of sexual relations and engage in them out of desire. But it is a small proportion. It seems clear that the competitive and exploitative attitudes on both sides make sexual relations a tense and uncertain matter, as far as gratification goes.

And when people talk about their standards of good behavior as opposed to the way things are, they speak of the same standards of premarital virginity, or at least of having intercourse only with someone the girl is going to marry, that the working class and middle class will talk about. So there is not really a separate set of norms. It is rather that the dynamics of the peer group community within the slum and the inability of the parents to maintain control over the boys and girls result in a situation in which sexual behavior is engaged in, taken for granted, but not approved, and, because of the exploitative elements, by and large not enjoyed by the girls at least.

Within the Negro lower class, the same pattern of exploitative sexual relations continues through marriage and plays a real part as the approximate cause of the high rate of marital disruption. Thus in part of our study where we interviewed women who were separated and asked them why their marriages had broken up, the reason given by most of them was the sexual infidelity of their husbands. As we did not talk to their husbands, we are probably underestimating the role of the sexual infidelity as a factor, undoubtedly the husbands would have some accusations of their own to make.

One over-all conclusion one can draw from an examination of sexual satisfaction in lower-class and working-class behavior is that sex is not one of the good things of life that are free; it is costly, too; and a satisfactory sexual relationship is very much a function of a generally satisfactory situation in life. The changes that we have seen in the situation of the stable working class since World War II, with increasing prosperity and affluence and increasing security, have been such as to shift—we know from research—the marital role relationships in the direction of this lesser degree of segregation. The effect has probably been to develop patterns of greater marital sexual satisfaction within the working class.

For the lower class, however, there has, unfortunately, been no change in socioeconomic situation, no relative improvement in their poverty vis-a-vis the larger society, but rather larger and larger concentration of people sharing similar miseries and, therefore, a deepening of the use of sexuality as a way of exploiting people for status and other kinds of gains.

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CULTURAL PATTERNING OF SEXUAL BEHAVIOR IN AMERICAN AND JAPANESE SOCIETIES

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This material is edited from the transcription of a verbal presentation, and is therefore informal. The details of the studies that I shall mention can be found in the published reports listed in the references.

There is in Japan a separateness in the activities of husbands and wives somewhat like that noted by Dr. Rainwater in the lives of lower-class American couples, a separateness that in the United States appears to be correlated with the lack of enjoyment of sex. With the Japanese, however, the separateness runs through the society in general and exists for reasons very different from those causing the separateness in the American lower class. It has different consequences, too, even though the structures may be similar.

A contrast is notable between Japanese and American cultures with respect to sexual behavior, child rearing, and indeed interpersonal relations of all kinds: the American culture emphasizes the individual's separateness, independence, ability to move—both socially and geographically—whereas in Japan the greater emphasis is on the interdependence of people. This cultural difference in what it means to be a human being in the two countries seems to have an effect on sex behavior as well as on other things. Sex is of less importance in the daily lives of the Japanese than Americans seem to think it is in their lives, if one can judge by what comes through in advertising, television, and general conversation.

In my work with psychiatric patients and with normal child development in Japan, the theme of interdependence emerged so strongly that I wished to learn something about the ways in which Japanese patterns of emotion contrasted with those of people in the United States. To study this question I used a picture-interview technique. The responses to three of the pictures have been analyzed (see Caudill 1962).

The first picture shows a man in bed on the floor, in Japanese style, being taken care of by a woman. The second one shows a young couple with the door into the next room pushed open, with bedding visible in the background. Unfortunately, the bedding is not mussed up, as it should have been. In the third, a young girl and presumably her brother a year or so younger are bathing together in a Japanese-style bathroom.

I asked the people interviewed to tell me stories about these scenes and to say what they felt about what they saw. I am not so much concerned here with describing the sample of some 100 people I interviewed as with talking about two patterns of responses that came out and how the patterns are interrelated across the three pictures.

The first pattern is that people looking at the first picture say that the man is mildly ill with a cold or a hangover and is staying home to be taken care of by his wife or his mother. In Japan there is a positive feeling about being ill. Mild illness is quite ego syntonic. People do not feel guilty about it; they go to bed readily with mild illnesses. The absenteeism for illness rate is considerably higher than it is in this country.

Looking at the second picture, people who rather like to be mildly ill see it as a pleasant, peaceful couple who are talking, for example, about how well their son is doing in school. Then the woman may say, "You are tired, dear, after a hard day's work; let's go to bed." But she means to sleep, not to have intercourse.

The same kinds of people, looking at the bathing scene, say, for example, "Oh that reminds me of how I used to bathe with my brothers and sisters." The Japanese word *kyodai* freely translated means siblings. You are not sure from the use of the word whether it means groups of boys or groups of girls or both. Someone will say, "It reminds me of the nice feeling I had about bathing with my brothers and sisters when I was young." The artist drew too fancy a bath for the ordinary Japanese home, so the person will probably add, "I wish I had a tub like that." Others will say it is nice to come home at the end of the day and get into a tub naked with another person, another adult, because you can relax and talk as you cannot when you have your clothes on.

What comes out of the interview responses, then, is that mild sickness is seen as positive, sex is underplayed, and bathing is an enjoyable sensual activity. This pattern of emotion responses accounts for 60 to 70 percent of the respondents. A minor variation in the case of the first picture is that some people say, "If he is hung over, he should be ashamed of himself. He is losing money and endangering his job. He ought to get up and go to work." These people do see sex in the second picture. For example, they see a married man who is visiting his mistress, and they remark with disapproval that now that he has satisfied himself he should go home to his family, but he probably won't. He will stay all night. These same people, upon looking at the third picture, will say, "I don't really like to bathe very much, except for getting clean. It is not a particularly enjoyable experience for me." This is usually coupled with responses about their childhood. If it is a woman talking she may say, "My father always wanted me to bathe with him when I was a young girl, and I didn't like to. My elder brother would say, 'Your father is asking for you, so you go ahead and bathe with him. So I would." Or a young man will say, "I did not like to bathe with my grandmother, because she always put the wash cloth over my face, and it sort of suffocated me. I have bad memories of that kind of thing."

Two themes are apparent here.[1] The first, and most frequent, theme might be thought of as "an emphasis upon nonsexual satisfactions" to be found in a variety of situations extending even to those situations in which sexual interest ordinarily would be expected. The second might be characterized as "a denial of pleasure and emotion" in an equally wide variety of situations, including specifically sexual ones.

I believe that these two general patterns of handling situations in which emotional impulses may easily be aroused are very common ones among Japanese. Why should this be so? In brief, many everyday-life events in Japan, whether for children or adults, offer greater opportunities than is true in the West for the gratification of simple physical pleasures in situations of close contact with other persons, as in bathing, sleeping arrangements, nursing care, child rearing, and so on. If sexual feelings were allowed to intrude into these events, this would complicate matters and the simple pleasures to be derived from these situations would be reduced. Japanese value such simple physical pleasures highly, and one way of assuring their continued existence is to ignore, to exclude, or in some way to isolate (as by joking) any sexual feelings that might arise. This mode of operation (or, for adults, one might even say this character structure) results in the general emotional pattern that is tagged here as "an emphasis upon nonsexual satisfactions." The "price" paid for adherence to such a pattern is the tendency to emphasize the nonsexual satisfactions to be gained from situations in which an interest in directly sexual matters might well be not only biologically but also socially appropriate. Thus, both biologically and socially, one would expect a man and wife, under reasonable conditions, to have a sexual interest in each other, but culturally this is not necessarily so.

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[1] The next three paragraphs are taken, by permission of the Aldine Publishing Company, from my paper (129-130) in the book named here. Reprinted from Robert J. Smith and Richard K. Beardsley, editors, *Japanese Culture*, Chicago; Aldine Publishing Company, 1962; copyright by Wenner-Gren Foundation for Anthropological Research, Inc.

So much for the first general pattern, which I believe is a major one in Japanese life. What about the second general pattern of "a denial of pleasure and emotion," which I think is a strong minor theme? Again starting from the frequency of occurrence in Japanese life of events that offer opportunity for the gratification of physical pleasure in situations of close contact with others, it seems likely that a significant number of persons (possibly for various combinations of genetic, social, and cultural reasons) have not been able to meet the emotional impact of such events by "an emphasis upon nonsexual satisfactions." For these persons, their sexual feelings do tend to intrude, insistently and uncomfortably, into such situations. Thus the "answer" is a general restriction of impulse gratification and a distaste for all too consciously recognized sexual matters. In the extreme this may result in an over-all denial of emotional feeling, whether positive or negative. These persons would fall into the second general pattern of "a denial of pleasure and emotion."

In a second study, we analyzed the sleeping arrangements of 300 families, each of them with a three- to four-month-old baby (some had older siblings as well), and about a third of them with a third-generation also represented—quite a complex number of bodies (see Caudill and Plath, 1966). Japanese will reply, jokingly, to questions about their sleeping arrangements with a reference to a Chinese character, *kawa,* that means a river—two vertical lines with a little vertical one in the middle—meaning that the child sleeps between the mother and father. The whole family sleeps together on quilts laid on the floor mats. In our study we controlled for density the number of rooms available for sleeping with relation to the number of bodies to be accommodated. All the following generalizations are true, even when one controls on density.

We found that the most usual pattern with a family consisting of mother, father, and a three- to four-month-old child, regardless of social class, is for the entire family to sleep together. Usually the mother is in the middle, with the baby on one side and the father on the other. If another baby is born two or three years later, the most usual pattern is that he is put on the mother's side and the older child sleeps between the parents. Since an average Japanese bedroom of six mats measures about nine by twelve feet, by the time there are four sets of bedding in it the room is pretty crowded. If, at this point, the family decides to divide its sleeping arrangements, the way this is done is very different from the way a Western family would do, with the mother and father in one room and the children in another. In the Japanese case, the father and the older child sleep in one room while the mother and the baby sleep in another, thus splitting the parents rather than the generation. The general feeling with the Japanese seems to be that a person would feel lonely if he slept alone. If there is a father's mother in the family, she will take the older child to sleep in her room, leaving the baby to sleep with the mother and father. This will go on until the children are about ten and often until they are thirteen or fourteen years old. About 80 percent of families in the study sleep in such patterns as these. For children between ages fourteen and twenty, in about 20 percent of the families, the children are still sleeping in two-generation groups; those who do not, usually sleep with a sibling rather than alone.

The average age at which women marry in Japan is about twenty-five and for men the average age is twenty-nine. With their first child, the pattern starts all over. The result, according to our study, is that in urban Japan today a person can expect to sleep in a two-generation group for approximately half of his life time.

Managing sexual relations in a crowded room like this is something of a problem. In a study of thirty American and thirty Japanese children from the ages of three to four months to six years, we asked our Japanese mothers how they made arrangements for sex. The usual answer was that they waited until the child was deeply asleep, then had sexual relations in the same room with the child. Sometimes there are signals between the husband and wife that the husband wants to have intercourse, and the mother will make some other arrangement; but usually it is just a matter of waiting for the child to go to sleep. Studies made by Shinazaki in Tokyo show that intercourse occurs less frequently than appears in the American data from the Kinsey investigations. The time taken also is shorter, and both foreplay and afterplay are shorter.

One of the things noticeable in the Japanese family is that, as might be expected from what I have been saying, there tends to be a close mother-child coalition in the family, with the father more removed from family life than he is in America.

The father leaves home about seven o'clock in the morning, spends a couple of hours traveling to work, leaves his work at five or six o'clock, and usually stops off to spend some time in a bar with his cronies. He may get home about eight to ten o'clock, or even later.

Before 1958, there was organized prostitution in Japan. It was a pleasant, cleanly-run thing, on the whole—very different from the furtive, sneaking-around kind of thing that often happens in this country. But in 1958 it was outlawed and the areas of prostitution were closed. This merely increased the number of bars, of which there are thousands in Tokyo, where men, regardless of their economic level, will stop off to have a drink with a bar girl with whom they have a kind of on-going personal, but not usually a sexual, relationship. The man who stops in to have a drink with the girl listens to her talk, which is part of her business; he has his ego built up by her telling him how good he is. He could arrange, with some effort, to go to bed with her, but usually he does not, because it would be too expensive. Instead, he leaves for home, riding about two hours on a train, and goes to bed according to the usual Japanese pattern. The bar-restaurant-prostitution set-up in Japan is quite asexual for the Japanese customers, contrary to the general Western belief, just as the family life situation also is.

In our study of mothers and infants in Japan and America (see Caudill and Weinstein, 1969), we took about 40 variables, 20 mother behavior and 20 baby behavior, and ran them down one side of a sheet with 40 columns across the sheet, and made time samples, one observation every 15 seconds. That gave us four observations a minute on the behavior of the mother and the baby. We did this over a ten-minute period with a five-minute break between sheets. We ended up with a total of 800 observations for every mother-child pair. We used 30 Japanese and 30 American pairs.

We used multivariate analysis of variance in which the dependent variables were the behaviors of mother and infant and the independent variables were culture, occupation of father (salaried or independent), and sex of infant.

Culture comes out to be by far the most important variable in this study. Sex of infant does not make any difference statistically at the age of three to four months, except that if one analyzes the American and Japanese data separately one finds that American mothers show more affection to their boy babies than to their girl babies, whereas this is not true with the Japanese mothers.

The main feature of these data is that the American baby is much more vocal—particularly more happily vocal—and more physically active than the Japanese baby, who is greater only in unhappy vocalization. There also is a pattern of positive correlation in the vocal behavior of American mothers and their babies. Mothers who are high in chatting to their babies have babies who are high in happy vocalization. This is not so for the Japanese cases. Apparently the American mother wants an active, responsive, self-assertive baby, and she gets him. The Japanese mother does more lulling, rocking, and soothing of the child because she seems to want a passive, contented baby, and she gets that. This pattern of greater vocalization, activity, and separateness for the American child also appears to be true from our further observations of the same cases in each country at the later ages of two-and-a-half and six years.

One of the nice things that have come out in our Japanese data at these later ages is that frequently a neighborhood girl two or so years older than our subject will, though she has not been formally asked to do so, take care of the child when he is out playing. We call this in Japanese the *nesan* (older sister) syndrome. It does not happen anywhere nearly as much with American children. There is, then, an expectation of being taken care of by a woman, which, especially for the boy, starts early and continues throughout his life. It comes up, I think, in adulthood in the custom of the man's stopping off at the bar, where he may refer to the bar girl as *nesan,* using the term of address for an older sister, even though the girl, of course, is not so.

There is, then, a different patterning of interpersonal relationships in Japanese and American cultures that I think makes for a considerable difference in the patterning of sexual behavior in the two countries.

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BEHAVIOR — NORMAL AND ABNORMAL: INTRODUCTION

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One of the greatest discoveries in connection with human behavior, said Dr. Lambo, is our knowledge of the modifiability of that behavior. We know that no human being is irrevocably committed to any specific form of behavior; any behavioral pattern can be modified one way or another. Apart from the so-called environmental factors, such as social and psychological influences, the effects of drugs, and even physical-ecological influences, we now know that the genes can be modified in various ways so that behavior can be altered.

Dr. Money brings to our consideration data derived from his clinical and experimental work in psychiatry and pediatrics and from anthropological field work that he has carried out among some of the aborigines in Australia.

SEXUALLY DIMORPHIC BEHAVIOR, NORMAL AND ABNORMAL

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MODIFICATION OF BEHAVIOR BY ENVIRONMENTAL ALTERATION OF THE GENOTYPE

In an earlier and more naive era of determinism in behavioral theory, if a particular feature of behavior was ascribed to heredity, it was thereby invested with an aura of being more genuine, durable, unmodifiable, and valid than behavior ascribed to environmental influence. To use the alliterative shibboleth of the nineteenth century, nature was assigned a higher scientific status than nurture in behavior theory. Today's experimental evidence requires a revision of such a naive point of view: it is actually possible environmentally to manipulate heredity so as to change the program of the genotype as expressed in the phenotype. The morphology of the organism can be changed, and its behavior as well. Sexually, the extent of the change may constitute a complete reversal of sexual dimorphism of morphology and of behavior.

This type of sexual reversal is well illustrated in the experiments of Yamamoto (1962) on the killifish, *orizeas latipes.* Yamamoto's experiments go one stage further, however, and demonstrate how environmental manipulation may change the very genotype itself and, with it, the genetic program for behavior.

Yamamoto exposed XY larvae of *orizeas latipes* (it is a viviparous salt and/or freshwater fish) to female sex hormone. Untreated, they would have differentiated as males. Treated, they differentiated as females. These XY females were, like normal XX females, able to breed with normal XY males and produce young: 25% of the second generation larvae were then chromosomally XX (female), 50% XY (male), and

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25% YY, which, if left untreated, would differentiate as males. If, however, the YY were exposed to estrogen, they would differentiate as YY females. These YY females would, in adulthood, be fertile, like normal XX females, but their ova would contain only a Y instead of an X chromosome. In the succeeding generation it was, therefore, possible to breed YY females with YY males: 100% of the resultant progeny differentiated as YY males (unless they were experimentally exposed to estrogen). In the reverse of this experiment, Yamamoto was able to produce XX males by treating XX larvae with male sex hormone.

What of the behavior of YY males? Hamilton and his colleagues (1969) at the Downstate Medical Center, New York, tested YY and XY males, in 14 matched pairs, in competitive mating for single XX females; the YY males were clearly dominant. They induced 137 of 155 spawnings. They gained higher scores for number of contacts with females; quivers, including those at spawning; moving in quick circles around the females; and number of seconds spent alone with females. YY males spent more time chasing the XY males than vice versa, and made more quick circling movements around the XY males. When one male chased another male, he would bite the pursued if he caught up with him. Biting was done almost exclusively by the YY males. Several XY males had their fins lacerated as a result. XY males tended to avoid YY males by remaining at one corner of the tank, near the bottom. One may presume, as Hamilton did, that the presence of an extra Y chromosome, rather than the loss of an X, was responsible for the YYs' increased mating dominance. Be that as it may, the chief significance of increased dominance, in the present context, is that it was produced by environmental alteration of the genotype itself.

MODIFICATION OF BEHAVIOR BY ENVIRONMENTAL ALTERATION OF THE PHENOTYPE

In the Yamamoto experiment, the first stage in altering the genotype was to alter the phenotype: the developmental male program of the XY genotype was suppressed by the addition of estrogen to the tanks in which the larvae had been spawned. A female program was thereby instituted instead, and the phenotype differentiated as female. At maturity, the breeding behavior was that of a female despite an atypical Y chromosome in 50% of the eggs.

Yamamoto's experiment is not the first in which the germ cells have been reversed from ova to sperms, or vice versa, while still retaining their reproductive fertility. Many years ago, Witschi demonstrated that overripe toad eggs all developed as morphologic males. Not only the genetic males, but also the genetic females had the appearance of males and produced sperms, but without the male sex chromosome present in any of them (Witschi, 1956, 1965). Witschi and his co-workers (Chang and Witschi, 1955, 1956; Mikamo and Witschi, 1963) also succeeded in producing a similar reversal of genetic sex in toads by implanting sex hormones into the developing larvae. In 1964, Turner and Asakawa made a first step toward achieving the same result in a mammal by transplanting the gonads of fetal mice into a host animal, so that the fetal testis turned the fetal ovary into an ovotestis in which spermatogenesis progressed to the point of secondary spermatocytes. Burns (1961) had in 1956 used estradiol in the fetal oppossum to convert a would-be testis into an ovotestis producing ovocytes.

It has not yet been reported experimentally possible to reverse the sex of differentiation completely from that of the genetic sex of the fertilized egg in mammals. Nonetheless the fish and amphibian experiments demonstrate how profound can be the reversal of everything pertaining to genetic sex: morphology, behavior, and fertility. These experiments require that one keep an open mind with regard to possible partial reversals of the expression of genetic sex in human beings, perhaps of direct relevance to sexual psychopathology, from causes as yet unknown.

The fish and amphibian experiments also point out a profoundly important principle in the theory of heredity versus environment (perhaps more appropriately designated as genetics versus environmentics). It is a principle that transcends the old dichotomy between nature and nurture by introducing the concept of the critical period. There is only a limited period during which a fertilized egg may be tampered with and forced to reverse the program for which it is genetically coded. After this limited or critical period, the die is cast and the program cannot be changed, or, having been changed, cannot revert.

MODIFICATION OF BEHAVIOR BY PARTIAL MODIFICATION OF THE PHENOTYPE: ANTIANDROGENIZATION

In mammals, though it has not proved experimentally possible to effect a complete sex reversal of the phenotype, it has proved relatively easy to reverse the phenotype of the external organs of sex. The principle involved may be simply stated as the principle of the masculine additive: add androgen at the critical fetal period when the external sex organs differentiate, and they will differentiate as male even in an XX fetus. Delete or suppress androgens at this critical period, and the external organs will differentiate as female, even in an XY fetus.

Experimental feminization of the male may be achieved surgically, by castrating the fetus *in utero*, and so depriving it of its own fetal testicular hormones. The alternative is pharmacologic feminization, which is achieved by injecting the pregnant mother with an antiandrogenic steroid that prevents the fetus from utilizing its own fetal androgen. The most successful antiandrogen so far discovered for this type of experiment is cyproterone. Neumann and his colleagues (1955, 1966) in West Berlin have produced XY rats that have completely female external organs. The gonads are testicles, undescended. The uterus and its fallopian tubes are not present as in a normal female, since the testes had been able in fetal life to secrete their Müllerian-inhibiting substance that prevented development of a uterus. Cyproterone does not interfere with this aspect of fetal development, only with the production of androgen.

Cyproterone administered to the fetus does not permanently impair the testes. They will secrete androgen at puberty. To permit the development of full feminine mating behavior at puberty, the testes are, therefore, removed and the animal is maintained on cyclic female hormones, estrogen and progesterone. Under these conditions, the mating behavior of these anti-androgenized males is exclusively feminine. The stud males of the colony recognize them only as females.

There is no ethical reason to antiandrogenize the human fetus. Thus, there are no experimental or iatrogenic examples of this condition in the human species.[1] An exact analogue does occur, however, in the syndrome of testicular feminization or androgen insensitivity. This condition of insensitivity of all of the body's cells to androgen is genetically transmitted, probably as an X-linked recessive or a male-limited autosomal dominant, in the female line. The biochemistry of the defect in cellular response to androgen has not worked out. This defect remains continuous throughout life. The affected individual is, therefore, not only born with a female morphology, but develops with an exclusively female puberty, except for absence of the menses due to absence of the uterus. It is self-evident that a baby with female external genitals will be assigned and reared as a girl. Genetics notwithstanding, she differentiates a psychosexual identity as a girl (Money, Ehrhardt, and Masica, 1968). Typically, in fact, she fits the feminine stereotype rather closely, with a negligible tendency toward tomboyism. In childhood play her maternal interests are strong and in adulthood, when she marries and adopts children, she is a good mother. In teen-age, her dating and romantic interests are inseparable from those of her age-mates, except that she has the emotional hurdle of coping with the knowledge of her sterility.

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[1] One possibility, however, made evident in the animal experimental work of Gorski (1968) is that barbiturates taken by the pregnant mother may antagonize the influence of androgen in the fetus, which is rather frightening in its implications with respect to the human male.

Since the differentiation of a gender identity in girls with the androgen-insensitivity syndrome is feminine, it is obvious that the Y chromosome in their genetic constitution is unable to express itself. Its expression is defeated first by the feminizing hormonal events of fetal life and, subsequently, by the events and experiences of being reared as a girl.

MODIFICATION OF BEHAVIOR BY PARTIAL MODIFICATION OF THE PHENOTYPE: ANDROGENIZATION

The counterpart of antiandrogenization of the XY genotype is androgenization of the XX genotype. Experimental androgenization is relatively easy to accomplish in animals, namely, by injecting the pregnant mother with androgen. A very considerable body of experimental literature has now accumulated on this topic, involving several species, including the rhesus monkey. When the dosage of androgen is sufficient, the external genitals of the female fetus are completely masculinized and the baby is born with a penis, foreskin, and fused scrotum (empty) instead of a clitoris, clitoral hood plus labia minora, and unfused labia majora.

There are two experimental traditions of androgenizing female animals. One relates directly to an interest in the behavior of masculinized females, the other to an interest in the pituitary control of estrous cycles. In briefest synopsis, the upshot of these two lines of work has been to show that partial fetal androgenization of estrous species, properly timed for the critical period, permanently unfeminizes the hypothalamic centers that govern feminine cycling of the pituitary and, at the same time, bizarrely disrupts the normal patterns of hypothalamically governed mating behavior appropriate to the phasing of the estrous cycle. In addition, fetal androgenization, especially when complete enough to result in the transformation of the clitoris into a penis, induces changes in the sexually dimorphic balance or frequency of behavior. Females with a penis gain scores on various frequency tests of behavior (behavior that may be in the repertory of both sexes) that deviate from the norms of their female controls in the direction of the male controls. These masculinized scores have been widely assumed to reflect a masculinization of the central nervous system.

From the human point of view, the most instructive androgenized females are the rhesus monkeys being raised at the Oregon Regional Primate Center (Young, Goy, and Phoenix, 1965; Phoenix, 1966). These penis-bearing females are, in brief, tomboys in their childhood behavior.

They gained behavior scores for initiating play, engaging in rough-and-tumble play, making threatening gestures, and adopting the mounting position in sexual play that were closer to the scores of normal control males than normal control females. The oldest of these animals have reached adolescence. They have normal menstrual cycles. With maturity, they appear to lose the masculine trend of their behavioral scores, but the evidence is still incomplete.

In human beings, the closest parallel to experimental masculinization in animals is the iatrogenic condition of progestin-induced hermaphroditism in genetic females. This rare condition first appeared about fifteen years ago when synthetic progestins were first introduced on the market and prescribed for the purpose of preventing threatened miscarriage. Certain of these synthetic products (all of which are closely related to biologically active androgens in chemical structure) proved to have a masculinizing influence on an occasional female fetus. In consequence, a baby is born with a greatly enlarged clitoris and partial fusion of the labia. The genital appearance in such an instance is the same as in an incompletely fused penis and scrotum in a genetic male. In general, the correct diagnosis is made at birth, and the baby is assigned and surgically corrected as a female. Hormonal puberty is normal, as for a female, and no endocrine treatment is necessary.

A study of the behavior of ten girls, in middle to late childhood and early adolescence with the partial masculinization of progestin-induced hermaphroditism (Erhardt and Money, 1967; Ehrhardt, 1969) showed them, like their monkey counterparts, to manifest a strong degree of tomboyism as compared with experimental controls. Such a girl judges herself to be a tomboy, as do also her family and friends, and is rather proud of it. Her tomboyism is defined, perhaps above all else, by vigorous expenditure of muscular energy and an intense interest in athletic sports and outdoor activities in competition with boys. It is not especially associated with aggression and fighting, though the tomboy will take up for herself when challenged. She rather scorns feminine frills and elegant hairdos in favor of utilitarian styles. In childhood, maternalistic doll play is perfunctory, as is the attitude toward anticipated mothering of newborn infants in adulthood. Career ambitions come first; they are commensurate with high academic achievement and high IQ, which seem to be a correlate of the syndrome. Career does not, however, exclude the anticipation of eventual romance, marriage, and pregnancy, to be combined with career. There is no tendency toward lesbianism; this kind of tomboy girl does not express the feeling that she ought to have been a boy or the belief that she would be better off if she could change her sex.

The tomboyism of progestin-induced hermaphroditism is fairly closely paralleled in another hermaphroditic condition, the early-treated female adrenogenital syndrome (Ehrhardt, Epstein, and Money, 1968). In this syndrome, fetal masculinization is the product of excessive androgen secretion by abnormal adrenocortical function. Treatment is with cortisone from birth. In an earlier generation, prior to the discovery of cortisone as the agent that would prevent excessive and premature pubertal virilization, teen-agers and women with the virilizing stigmata of the adrenogenital syndrome had lesbian fantasies and/or desires significantly more often than do affected individuals of the current generation who have been successfully treated from birth. In the older patients one presumes that, whatever the masculinizing effect of fetal androgens on subsequent behavior, it was augmented by the continuing rapid masculinization of the body and its appearance after birth. The remarkable finding among these older, heavily masculinized patients, however, is that only a relatively small proportion did develop lesbian desires and behavior.

MODIFICATION OF BEHAVIOR BY SEX ASSIGNMENT AND REARING

The foregoing hermaphroditic findings all pertain to modification of the morphologic and behavioral phenotype of sex by events in the prenatal environment that interfere with genotypic expression. Hermaphroditism is of further scientific value in showing that the postnatal environment also may exert so strong an influence as to counteract the normal expression of the genotype in the differentiation of gender identity and gender role. The key evidence derives from matched pairs of hermaphrodites, each with the same ambiguity of external genital appearance and each with the same diagnosis, but one assigned and reared as a boy, the other as a girl. That such disparities can and do occasionally occur is not by design, but by reason of imprecise diagnosis, or of different traditions of deciding the sex of assignment in cases of maximum ambiguity.

Notwithstanding variations in such traits as maternalism, tomboyism, dominance, and responsiveness with genital arousal to visual and narrative erotic images (a masculine trait) versus haptic sensations (on which the female is dependent for genital arousal), the typical finding in matched pairs of hermaphrodites is that the gender identity and gender role differentiate in conformity with the sex of assignment and rearing. This conformity is of such strength that it may withstand even the ambiguous or contradictory appearance of the defective sex organs and the bodily effects of a contradictory hormonal puberty, namely, ugly virilization in a girl and partial androgen insensitivity with breast development, absence of the beard, and erectile inadequacy in a male. Ideally, of course, it is infinitely preferable that these contradictions do not exist, and that the growing boy or girl has no paradoxical information from the body as to his or her gender.

Even though contradictions of the body can be effectively circumvented in the establishment of a gender identity, they constitute a severe hazard, not only because of their visible meaning to the child, but also because of their meaning to all others who have dealings with him (or her). Ambiguity and uncertainty in the minds and actions of other people (children as well as adults) are probably the single most important factor in preventing a child from developing a consistent and unitary gender identity. Continuously made aware that something is wrong sexually, a child does not easily tolerate the cognitive dissonance of his ambiguity.

It is rare for a hermaphrodite to differentiate a hermaphroditic and ambiguous gender identity. Instead, a resolution of ambiguity is achieved by way of the principle of opposites. There are only two acceptable alternatives in sex: male and female. If one's assigned status as either appears unsatisfactory or obviously erroneous, then the simple solution is to consider a change to the other. Whatever the pros and cons, the alternative at least has the virtue of not yet having been proved wrong and unsatisfactory. All told, there are not very many hermaphrodites who develop a conviction that they should have their sex reassigned. Among those who do, there is no consistent relationship between the genetic sex, the assigned sex, and the reassignment desired. For example, a genetic female hermaphrodite assigned as a girl may feel compelled to live as a boy, whereas another of the same diagnosis and assigned as a boy may have the opposite conviction.

Cases of hermaphroditic sex reassignment thus may show the same contradiction between sex-chromosomal status and gender identity as is manifested in some unreassigned hermaphrodites. In both instances, the evidence indicates that experiences of sex assignment and rearing can interrupt and reverse the orderly progression of events that ensure for the majority of the human race a perfect positive correlation between sex-chromosomal status and gender identity. The correlation between sex chromosomes and sexually dimorphic behavior then becomes perfectly negative.

GENETICS AND GENDER IDENTITY

For generations mankind has lived with a popular and scientific folklore that something so intensely and personally unnegatable as one's own sense of gender identity must, in some way, be preordained in the genes. By the same token, anomalies of gender identity, as in hermaphrodites who seek sex reassignment, in anatomically normal transexuals who do the same, and in homosexuals of the more mundane varieties than transexualism, also would need, theoretically, to be preordained in the genes. All the experimental evidence, however, from sex-reversed fish to human hermaphrodites shows that such a simple-minded theory is untenable.

The theory needed in its place is one that sees gender identity as the end product of a sexually dimorphic development sequence. The sequence begins with the dimorphism of the genetic code as manifested in the XX and XY chromosomal dimorphism. From the genetic code, sexual dimorphism is translated into the dimorphism of embryonic differentiation of the gonads, which, through their hormonal secretion, in turn differentially regulate the dimorphism of first the internal reproductive structures and then the external genitalia. At the same time in embryonic life, gonadal secretion dimorphically regulates the differentiation of structures in the brain, specifically the hypothalamus, that in turn will regulate the sex-related functioning of the pituitary. In all probability gonadal secretion at this same time also dimorphically regulates other structures of the brain that will eventually be involved in the regulation of certain aspects of sexually dimorphic behavior, namely, those aspects that are phyletically widely distributed (like motherly attentiveness to the newborn or coital postures and movements).

At the commencement of each stage in the sequence, the organism is, in effect, bipotential for the next phase of differentiation. In the normal course of events, this bipotentiality will never show itself. Therefore, it must be inferred from the known clinical examples of anomalies in dimorphic differentiation and from cleverly conceived experiments designed to demonstrate it.

Despite whatever sexual dimorphism may already have differentiated in the central nervous system, the human organism at birth is still largely bipotential for dimorphism of gender-identity differentiation. More simply said, the individual's gender identity and role (identity is the subjective experience of role, and role is the enactment of identity) will differentiate in response to and in interaction with stimuli encountered after birth.

This state of affairs is analogous to the development and differentiation of bilingualism in a child with two native languages. At birth, the child has, like other members of the human race, differentiated a brain programmed to use language, subject to the proviso that it encounters language postnatally. When this brain encounters the vocal signals and auditory oscillations of two languages, it must accomplish the incredible feat of coding the two separately, which, incidentally, is easier if the contextual cues are different, as when the two languages are used by different people exclusively. Eventually the child is able to use each of the two languages, each as a completely self-contained and autonomous system. It is possible to use the one correctly only by excluding the other. The differentiation of a gender-identity role resembles bilingualism in that it requires a brain to code two systems, the male and the female. Whereas there are only certain cases of bilingualism in which one of the languages is censored or avoided, in the differentiation of gender identity one of the systems must routinely be negatively coded and suppressed. The other is positively coded and actively in operation. Thus one may say that a boy knows how to be a boy because he knows also how not to be a girl. The establishment of gender identity and role is an active process of differentiation (with total success not necessarily guaranteed). The analogy is with the painter and his canvas, not with the photographic image absorbed on a blank paper. The latter, unhappily, is the one tacitly assumed in almost all social-learning theories of sex-role formation through identification with role models.

Because the absorbent-sheet model does not apply, it is not so important that a child have a perfect model for gender identity and role as that, in the sum-total of his experiences, the masculine and the feminine are understood and experienced as different and not as equivocal or the same. What is masculine and what feminine is less important, in any given household and community, than the fact that they can be distinguished, even though extensive overlap is acceptable. This is the principle of dimorphic signals.

Gender identity and role are in an active phase of differentiation during the period of late infancy and early childhood. The differentiation process would appear to be a rather delicate one, rather easily subject to disruption, perhaps more so in boys than in girls. The disruption need not be self-evidently associated with sex. I suspect it may be, among other things, a sequel to insufficient infantile tactile stimulation (Harlow's 1965 monkey experiments are a prime paradigm here); to too much personal closeness or overcrowding; to life-threatening illness or death of a close family member; to family feuding with the child as pawn; to atypical early exposure to sexual activity or play of excessive dramatic impact, perhaps with insufficient prior opportunity for routine sexual play in childhood; and to equivocation or ambiguity in the parents' expectancies of masculinity or feminity for their sons or daughters. A brain lesion may disrupt gender-identity differentiation, but this effect is more generally seen in adulthood, as a deterioration, than in the formative years.

These various environmental events—and others yet to be identified—that may disrupt gender-identity differentiation may also leave permanent sequelae in the form of aberrations of sexual behavior. One cannot say, however, in this instance that environmental events modified genetic expression. The genetic code does not exhaustively program gender identity and role as male or female in the human species. The program in the genetic code spells out only a readiness to differentiate a gender identity and role (or in certain cases, like the XXY and XYY syndromes, perhaps impairs readiness). The details are, as in the case of language, programmed in the social code of interaction and learning. Herein lies ample opportunity for error of differentiation—for all the aberrations of psychosexual function, in fact. This is a manifestation of the range of the versatility and flexibility of response in our species, as compared with the stereotype in other species. It is a feature of man's phyletic heritage that environmental influences of the genetics of our behavior are vast in scope—more vast than in any other species.

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MALE HOMOSEXUALISM AND AFRO-BRAZILIAN RELIGIONS: A PRELIMINARY REPORT

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To test some generalizations about the occurrence of homosexualism among priests and followers of African-derived religions in Brazil (Landes), a research project was devised and carried out among the Afro-Brazilian cult groups in Recife, Brazil, during a period of two and a half months at the beginning of the current year. The cult groups whose cooperation was secured were classified in six categories to deal with the main variables considered: traditionalism, syncretism, and the interlinked types of leadership (males, females, and homosexuals).

Ten men in each of the six categories were interviewed personally by the author with the free-question technique, thought to be the only one adequate for the purpose in view of the groups' long-established tradition of discretion, secrecy, and reticence about personal and cult matters.

Twenty-three questions of relevance were asked in more or less sequential order in tape-recorded interviews of from twenty to thirty minutes. Besides general information, such as age, civil status, occupation, education, place of birth, and so forth, the essential topics focused upon were: family and social experiences; how, when, and why they associated with these groups, their patron gods and other spirits, if any; physical and personality mythical characteristics of those entities; relation between the personality of the gods and that of the follower, if any; personal opinion of male and female attendants at the cult; opinion of homosexuals and their attendance at the ceremonies; the experience of possession; rank in the cult hierarchy.

In the interview sessions held at the cult houses with followers summoned by their leaders, the Draw-a-Person projective test (Machover variety) was given independently by our assistant psychologists Carmen

Maria Mota Cardoso and Norma Lucia do Amaral Cesar, who also evaluated and interpreted the drawings.

The author is well aware of the reservations some specialists have about this technique. However, we note that after a retest experiment, Stanley R. Graham came to the conclusion that the test "continued to reflect a consistent picture of the self-image, despite attempts on the part of subjects to disguise or conceal what they knew or imagined to be significant details relative to weaknesses in their own personalities," (Graham, S. R., 1956). S. Starr and F. L. Marcuse, also examining the reliability of this test, concluded that, at least for males, the sex of the first-drawn figure was a reliable factor, other things like perspective, position on page, incompletions, height of the figure, and ratio of head size to height of figure being reliable for both sexes (Starr and Marcuse, 1959). Finally it should be said that the validity of the test has been discussed to a great extent by Karen Machover herself (Machover, 1957).

CASE STUDIES

Considerations of time and space limit our analysis to the study of cases in which severe sexual maladjustments, such as homosexuality or homosexual trends and problems of sexual adequacy, were detected. The cases thus selected number 34, or 57 percent of the subjects interviewed; they will be summarized below. The remaining cases and other data collected in this research will be reported elsewhere.

I Homosexualism

Four cases were the homosexuals screened through the interview and testing sessions. Three of them were under thirty years of age, one forty-five years old, all affiliated, as would be anticipated, with groups led by homosexuals. They show maternal dependence, introversion, immaturity, egocentrism; some are exhibitionists, narcissists, mannerists, often guilty, timid, or suspicious, and with great difficulty in relating to the female figure.

Case 24. Age 45; is timid, insecure, dissatisfied with ego. Needs social acceptance or appearance of being socially powerful, dominant. Tends toward exhibitionism. Ambitious, aggressive (verbally, too), with guilt feelings from his aggressiveness. Has difficulty with social relationships. Is mother-dependent. Mother-image is hostile and dominant. Shows sexual ambivalence, mannerism, maladjustment, and effeminacy. Ogun, his patron-god, is ambivalent. He feels congenial with Oshun, one of his gods, who is described as a girl, an adolescent, "gay." Shango, his second god, is a "strong man." Experiences possession. Joined the cult ten year ago because of physical illness and economic needs.

Case 54. Age 24; an introvert, infantile, egocentric, narcissistic. Has conflict in relationships and with authority. Shows infantile behavior, need for approval, sensitivity, fantasy, aggression, insecurity, exhibitionism, social and emotional maladjustment. Father died when he was young. Mother-image is powerful, aggressive, rejecting; experiences feeling of rejection by mother, notwithstanding his marked dependence upon her. Began working as domestic servant at 12. Displays confusion over sex role, mannerisms, homosexual panic, somatic projections. Is confessed homosexual. When 15 year old, left home and went to live at a cult house led by a notorious homosexual. Questioned about his homosexual affairs, he said: "I want a lover, but I never meet with response." Two of his gods (Odé and Shango) are punitive, while the third is "gay" (Oshun). Experiences possession. Joined the cult 9 years ago after spontaneous possession. Had attended spiritualist sessions before.

Case 59. Age 19; first drew a female figure. Is egocentric, immature, dependent, insecure, suspicious, aggressive in social relationships. Has guilt feelings because of aggressiveness. Shows repressed internal rebellion against society. Lacks stability; has difficulty at harnessing his impulses. Eludes somatic problems. Has difficulty with relationship to feminine figures. Is confessed homosexual. Recalls his father as authoritarian and rude, his mother as equally rude and often punitive. Is attached to and identified with mother. Has difficulty in relationships with other sex. Liked his homosexual experiences. Lives with his parents and seeks their approval. Says his gods (Shango, Oshun, Ogun) are kind and protective. Never experienced possession. Joined the group for psychosomatic ailment. An uncle is also a cult member.

Case 60. Age 28; first drew a female figure. Is immature and infantile, with strong narcissism and egocentrism. Not very intelligent. Shows social and emotional maladjustment. Is timid, insecure, evasive, and sensitive in social contacts. Shows compensatory arrogance and disdain as aggression toward society. Is vain and exhibitionistic, hostile to the feminine figure, effeminate. Has conflict with regard to sex identity. Experiences depression, feeling of inferiority; is confessed homosexual. Recalls his father, who died when he was 6 years old, as tall, lean, peaceable, not punitive. His mother also is tall, but fat, "nice." Ran away from home because his mother and sister objected to his passive homosexuality. Lives alone. Shows identification with and dependence upon his mother. Said that "women do not give pleasure; men do." His patron deity (Oshun) is "good, gay"; Ogun is "severe"; Yemanja is "good," also "gay." Experiences possession. Joined the cult while young (father was a *babalorisha*) because of physical ailment.

II Difficulty with Sex Identification

To draw a female figure first on the D-A-P test, according to Starr and Marcuse, quoted above, is a sign of conflict and confusion about one's sexual role. It should be recalled also that Thomas Colley developed a theory about sex identity to the effect that the proposed concept of *psychomode* involves the sociopsychological factors of sex identity that are so important for the adequacy of sexual behavior. Normal or abnormal sexual behavior, therefore, depends largely upon the social interactions established by the child from early infancy and upon the correspondent specific reactions of parents, mainly the sexual role they assign him (Colley, 1963). John Money and the Hampsons demonstrated some years ago that in 100 hermaphrodites (with rare exceptions) their sexual psychology was independent of the biological factors and was found rather consistent "with their sex of assignment and rearing, even when the latter contradicted chromosomal sex, gonadal sex, hormonal sex, the predominant internal accessory reproductive structures, and the external genital morphology." (Money, Hampson, and Hampson, 1957). Finally it must be pointed out that Sidney Levy concluded from his assessment of the D-A-P test that those subjects who draw a female figure first show "sexual inversion, confusion of sex identification, strong attachment to or dependence on parent of opposite sex, strong attachment to or dependence on some other individual of opposite sex." (Levy, 1950).

These conclusions, derived from experimental evidence, suggest the importance of correlating difficulty with sex identification revealed by some of our subjects with other features, such as their parents' images, their attachment to or dependence upon one of them, and their types of personality as revealed by the D-A-P test and the interview sessions.

Sixteen subjects who drew a female figure first show different features in their imagery of father and mother figures and in their attachment to their mothers.

a. *Syncretic groups:* Subjects in this category, whether the groups were directed by male, female, or homosexual priests, indicate consistently that their fathers were strong, punitive, frightening, and rejecting (in a few cases insignificant or neutral figures), while their mothers were calm, protective, nice (in some instances also punitive, strong, authoritarian but possessive). Their personalities were in most cases egocentric, narcissistic, introverted, with a difficult relationship with the female figure, aggressive and sensitive in social relationships, sexually deviant, with conflicts over virility, insecurity, and confusion over sex identity. They showed homosexual trends.

Case 34. Age 19; narcissistic, introverted, egocentric. Is insecure, sensitive, timid, evasive, and aggressive in social relationships. Is vain, needs social dominance. Has frustrated intellectual aspirations. Shows difficulty in relationship with the female figure, hostility toward women, conflict over virility, deviant sexual behavior, and need for virile affirmation. Says his father was very severe, *although he was one year old when his father died.* His mother is calm and just, but "as severe as my father" (ambivalence). He lives with her, likes her company, and is submissive to her. Wishes to remain single. "Do not have [sexual] pleasure with women; do not like them," but has never had sexual intercourse. His patron god, Ogun, is "good," physically strong. Experiences possession. Joined the cult two years ago, attracted by the drumming (?). Also felt unlucky at that time and suffered from cephalalgia.

Case 39. Age 55; egocentric, immature, narcissistic. Has conflict in social relationship, with aggressiveness, lies, parasitism. Unemployed. Ambitious, with need for affirmation and power. Is insecure, dependent, with strong identification and with dependence upon the mother figure. Shows confusion over sex-identity, homosexual conflict, voyeurism, guilt feeling over masturbatory habits. Says his father was severe; deserted his mother when he was an infant. His mother is harsh, punitive, short, sturdy; nonetheless he feels a strong attachment to her and seeks identification with her. Both his gods (Ogun, Yansan) are "calm"; also his three Indian spirits are "nice." Experiences possession. Joined the cult 11 years ago because of physical illness.

Case 41. Age 32; psychosexually immature, with traits of introversion, egocentrism, narcissism. Is malajusted in social relationship; shows insecurity, sensitivity, aggression. Vain and exhibitionistic, he needs support and praise. Shows a tendency to lie and be ostentatious. Has difficulty in relation to the female figure and with sex identity. Needs virile affirmation. Shows sex maladaptation. Was married for 12 years; had no children. Says his parents are both authoritarian and punitive. Is attached to his mother. So narcissistic that projection is blocked. Shango, his patron god, is punitive, harsh. Yansan, his second god, once burned his tongue as punishment. Oshun is "gay." Resists possession by this deity because it dances in "too sexy" a manner. Experiences possession. Joined the cult because he was unemployed.

Case 43. Age 70; mentally weak, regressive, vegetative. Has low energy and a feeling of dwindling. Lacks control of impulses and reactions. Social relationships are superficial. Lacks emotional tone. Has difficulty with sex identity. Shows senility and organic depression. Was twice married. Says his father was severe, liked to punish and correct his children and never tolerated any disobedience. His mother was tall, strong, less punitive than his father. He was afraid of his father but attached to him. *His father died* when he was 8, his mother when he was 12. Identified with the female figure. Shango, his patron god, is peevish. Experiences possession. Joined the cult while taking his wife to a spiritualist center for treatment of a physical ailment.

Case 44. Age 56; shows introversion, narcissism, vanity. Is conflictive in social relationship, timid, insecure, exhibitionistic, highly sensitive, superficial and emotionally neutral in contacts. Has guilt feelings over probable dishonesty. Related to the mother figure. In the relationship with the feminine figure, shows sex difficulty, devaluation of sexuality, and debasement of women. Worries about expressing masculinity in compensation for homosexual conflict and difficulty over sex identity. For a time, was sexually "wild"— had three lovers. Married at forty. Sees his father as "neither good nor bad," but punitive, harsh, and violent. His mother is protective; she appeased her husband and sided with the children. Has feelings of rejection by his father. His guilt feeling is introjected and rationalized: "I had a bad temper." Devalues women and overvalues his mother. Patron god, Ogun, "ironhanded"; Eshu, "bad"; Yansan, punitive. Experiences possession. Joined the cult because of familiarity (attended the public ceremonies).

Case 48. Age 61; egocentric, narcissistic, introverted, primitive, regressive. Experiences feeling of weakness, insufficiency, and disgust with his body. Shows open aggressiveness in relationships, bad temper. Shows mistrust, sensitivity, with difficulty in relationships with women, is sexually inadequate and disordered. Shows senility. Says his father, now dead, was severe, harsh, authoritarian; his mother was less severe, more calm, peaceful. With flagrant ambivalence, later represents her as hostile, aggressive, and dominant. Shows attachment to mother, with ambivalent identification with both father and mother. Has difficulty in relationship with the female figure. Married at 35. His patron god, Oshun, is severe, temperamental. Is evasive about other deities (Abaluwaye, Yansan). Experiences possession. Joined the cult 13 years ago because of physical illness.

Case 49. Age 53; shows psychosexual immaturity, egocentrism, narcissism. Needs power and domination in social relationships. Is sensitive, insecure, timid. Shows exhibitionistic traits, repressed aggressiveness (also verbal). Has conflict in relationship with the feminine figure. Shows hostility toward women, whom he sees as dominant. Is dependent on his mother. Has homosexual conflict and homosexual tendencies, with need for virile affirmation. Was educated at an orphanage from the age of 12. His father was an Indian mestizo, tall, strong, frightening; he died when the boy was 8 months old (?). His mother was calm. He was raised by an aunt, also very "calm," who punished him when he deserved it (?). His rationalization is, "when I was young, I was too perverse." Shows attachment to and identification with his aunt. Said of the female figure: "gives little [sexual] pleasure." A widower, he is married for the second time, but shows hostility to his wife. He likes his "mother" better, "because a mother is forever, while a wife may be here today, gone tomorrow." His patron god, Eshu, is severe; other gods (Ogun, Oshun, Shango) are "reasonable." Experiences possession. Joined the cult 20 years ago (parents were cult initiates) because of economic need.

Case 52. Age 20; shows psychosexual and emotional immaturity, egocentrism, narcissism. Has conflict in social relationships. Needs social approval. Has compulsion to appear socially powerful, dominant. Is aggressive in his contacts, also sensitive, suspicious, superficial, with little affectivity. Shows hostility to and rejection of the feminine figure. Is single, reserved, reticent. Has conflict over sexual inadequacy. Shows homosexual tendencies, confusion about sexual role, ambivalence and sexual tension, homosexual panic, traits of schizophrenia. Says his father is strong and severe; his mother is calm. Feels more attached to his mother and is afraid of his father. His patron god, Abaluwaye, is said to be generous (?). Never experienced possession. Joined the cult three years ago while escorting his sister, who was an initiate.

Case 55. Age 20; psychosexually infantile, emotionally dependent. Shows traits of introversion, narcissism, intellectual inhibition. Although socially maladjusted, shows energy, stability, optimism in relationships. Is mother-dependent. Shows inadequate and phantasistic relationship with the female figure. Has difficulty in heterosexual contact, confusion over sex identity, insecurity over own virility. His father is dead. Says his mother is calm, punitive, but just. Wishes her the best in the world and centers his ambitions around her figure. Also wishes to live long to support her; gives her anything she needs. Hopes she will stay alive for a long time. Is submissive to her, and, being single, lives with her. Patron and other gods "calm," but he distrusts Shango because he is a lover. Never had possession. Was taken to the cult while an infant for a physical illness.

b. *Traditional groups:* Subjects from the traditional houses said their mothers were the dominant figures, while their fathers were submissive, neutral, or absent. The only exceptions were two cases (11 and 18) in which both parent figures were equally punitive or neutral. The personalities of these subjects are equally introverted, narcissistic, egocentric, immature, with insecurity, showing aggressive reactions and difficult relationships with the female figure. Attached to and dependent on their mothers, they also show homosexual trends, homosexual panic, or sexual inedaquacy.

Case 1. Age 33; is dependent, autopunitive, of low intelligence. He is socially and emotionally maladjusted, ambitious, seeking power and social dominance. He is timid, unstable, insecure, for which he compensates through aggressiveness. Experiences guilt feelings as a consequence. Is immature, with sexual inadequacy and sex difficulties, being unsure of his sex identity and confused over his sexual role. His mother is active, authoritarian, punitive. His father was bland, peaceable, and did not like to beat his children. Left home at eighteen and accepted a job as a cook. He is married to a woman weighing 200 pounds; says he "likes them tall and sturdy." Claims similarity of temperament with his mother, to whom he is attached. His patron god, Oshala, is "calm"; Yemanja is peaceful and kind. Never had possession. Joined the cult 10 years ago because of economic need. First went to a spiritualist center.

Case 3. Age 54; introverted, immature, narcissistic. Shows conflict in social relationships, with lack of confidence in contacts. Needs domination and social power. Insecure, he reacts with almost psychopathic aggressiveness. Shows signs of exhibitionism. He is dependent on the mother figure, but wishes for freedom from his mother's domination. Is sexually ambivalent and confused, with homosexual trends. Says his deceased father was calm and kind. Sees his mother as strong, harsh, "perverse." She mistreated her children. Though raised under her "severe" and "perverse" discipline, he lives with his mother, to whom he is attached. Shows identification with the mother figure. His patron god, Shango, is said to be impulsive. He is reticent about Oshun and Yansan. Never had possession. Joined the cult 6 years ago while escorting his wife, who is a cult initiate.

Case 5. Age 51; introverted, egocentric, primitive, immature. Has feeling of ego dwindling. Shows low energy level and evades organic problems. Has difficulty in social relationships, with reactive aggressiveness. Shows instability and insecurity. Is attached to the mother figure. Shows sexual inadequacy, sexual ambivalence, need for virile affirmation, problems of senility. His father was severe, but calm and less punitive than his mother. His mother was severe and authoritarian. He left home at 12 and went to his grandfather and uncle's home. Identifies with his mother; is afraid of his father. Married when 30 years old. His patron god, Oshun, is punitive; Ogun is seen as good tempered. Experiences possession. Joined the cult through familiarity and in consequence of physical illness.

Case 11. Age 48; is introverted, narcissistic, egocentric, immature. Has difficulty in social relationships, with reactive aggressiveness. Needs muscular force to compensate for weakness. Is timid, insecure, exhibitionistic. Shows conflictive relationship with the feminine figure. Had a stepfather. Is dependent upon the mother figure. Shows confusion and inadequacy in the sexual area, with compensatory wish for virile affirmation. Shows homosexual panic and marked degree of resistance to the recall of his parents' figures. Denied any memories of his parents, but said that both were punitive. Showed difficult relationship with the feminine figure. His patron god, Oshala, is kind; Shango is restless. Is reticent. Never had possession. Joined cult because of familiarity since his infancy (parents were initiates).

Case 18. Age 68; introverted, narcissistic, egocentric. Shows superficial contact with low affective tone and guilt feelings resulting in aggressiveness. Presents evasive attitudes and shows sensitivity in relationships. Tends toward exhibitionism and ostentation. Is insecure, with attachment to the mother figure and strong dependence on her. Has difficult relationship with the feminine figure. Has sexual difficulties and worries. Also shows ambivalence. His father is said to be good, old. His mother also is good, modest. Says he married to escape this world's misdeeds. Good relationship with both parents. His gods, Ogun and Yemanja, are both good tempered, not punitive. Never had possession. Joined the cult after consultation for his wife, who was ill.

Case 22. Age 51; is immature, emotionally dependent, with traits of egocentrism and introversion. Has weak control of impulses and reactions. Is ambitious, seeking social power and domination. Shows conflict in social relationships, insecurity, inferiority feelings, suspicion, sensitivity; makes contact with difficulty and with reactive aggressiveness. Started working in agriculture at 10. Shows indications of an authority-submission problem. Is aggressive toward the feminine figure, with attitudes of debasement. Has difficulty over sex identity, with feelings of sex inadequacy, insufficiency, impotence. Show homosexual trends. Says that he looks like his father, who was peaceable, kind to his children. The mother is authoritarian, severe. He is attached to both parents, with whom he identifies. Shows tenderness to them. Was divorced once. Is reticent about his gods. Experiences possession. Joined the cult 10 years ago because of nervous depression and compulsion to suicide.

Case 30. Age 26; shows introversion, psychosexual immaturity, egocentrism, narcissism, parasitism, moral rigidity. Is timid, evasive, sensitive, insecure, aggressive, dependent, exhibitionistic, with power and domination needs in social relationships. Bestows authority upon women. Shows hostility, tension, evasion. Has sexual phantasies related to the mother figure. Shows identification with and dependence upon the mother. Shows sexual asthenia, ambivalence, autoeroticism, confusion over sexual role. Is married to a woman 9 years older than himself. Says, "I would love to look like my mother, but everybody says that I am a copy of my father." His mother is said to be punitive, dominant, strong. Says his father is neutral and spends most of his time away from home. He lives with his parents. Is reticent about his gods (Oshun and Shango). Unemployed. Experiences possession. Joined the cult 7 years ago because of physical illness.

Two modal types of family constellation emerge from the foregoing case studies. In one, common to the members of the syncretic groups, the father is pictured as severe, authoritarian, punitive, while the mother is often calm, kind, protective. The only exceptions are one in which both parents are punitive, one in which a deserted mother is also punitive, and a case of a lonely mother who is lenient. In the other type, derived from the study of cases from the traditional groups, fathers tend to be absent (by death or other reasons), or neutral, calm, not punitive, while the mothers are mostly authoritarian, strong, dominant, punitive. The only exceptions are a case where both parents are punitive and another in which both parents are equally lenient. In spite of the difference, subjects of both types of family constellation developed the same attachment to and dependence upon their mothers, difficulty with sex identity, and ambivalence or difficult relationships with feminine figures. They all present sexual problems related to the sex role, homosexual trends, and sexual inadequacy.

Parental interaction and influence are recognized to be of paramount importance in the psychosexual orientation of the individual, since the biological and constitutional theories of sex identity were dismissed by scientific evidence like that reached by John Money and others. That is also the conclusion of Irving Bieber from his comparative study of homosexuals and heterosexuals submitted to psychoanalytic treatment: "Male homosexuality is primarily a disorder of sexual development in individuals toward whom highly pathological parental attitudes and behavior have been directed," (Bieber 1967), although he admitted the existence of biological "steering mechanisms" aroused by olfaction that may normally "ensure the heterosexual direction of object." (p. 966). According to the same author, the parental constellation "most likely to produce a homosexual or a heterosexual with severe homosexual problems was a detached, hostile father and a close-binding, overly intimate, seductive mother who dominated and minimized her husband." He goes into details indicating that this mother, besides being overly intimate, is also possessive, dominating, overprotective, demanding, as well as demasculinizing of her child. Puritanical and sexually frigid, "she interfered with her son's heterosexual interests in childhood and adolescence . . . babied him and hindered his participation in normal activities and the rough and tumble of boyhood." (p. 968). She may also be "detached, rejecting, and overtly hostile." The son's attitudes toward her "included submissiveness, overeagerness to please her," respect, admiration. The son turns to her for protection and sides with her in family quarrels. Few homosexuals "hated or feared their mother." On the other side, the father, according to Bieber, "was usually detached, unaffectionate and hostile to his son," who may be his least-favored or scapegoat child to whom he gives little of his time. The homosexual hates and fears his father and lacks respect and admiration for him.

The attachment to his mother and the hostility to his father coexist in the homosexual with a morbid resolution of the oedipus complex. The individual who fears and hates the "sexually destructive" or "weak and inadequate" father and later projects sentiments of dependence, competitiveness, and hostility upon other males comes to see heterosexuality as threatening, conflicting, and inadequate. The personality pictures of the subjects whose case studies are referred to here, whether overt homosexuals or males thwarted in their sexual identity, are a result of the conflictive situations they have experienced. Therefrom come the introverted, immature, infantile, narcissistic, egocentric, mother-centered, father-hating, aggressive, socially inadequate, and sensitive personalities with ambivalence toward the sexual roles and overt or covert homosexuality that loom large among our case studies.

Seduction, popularly held as an important factor in homsexuality, is dismissed by Bieber with conclusive evidence (Doshay 1943), although the homosexual "entourage" may not be minimized, given its notorious supportive and recruiting role. It should be recalled along this line that the only overt and confessed homosexuals in our case studies were found in houses directed by homosexuals.

III Problems in Sexual Adequacy

Subjects with problems in sexual adequacy are fourteen in number. Five among them were alcoholic addicts with deeply disturbed personalities, whose case studies will be omitted for lack of space.

SEXUAL INADEQUACY AND HOMOSEXUAL TRENDS

Case 16. Age 30; introverted, narcissistic, immature. Shows aggression in contacts with environment, insecurity, instability, and need for power and dominance. Left school because of lack of discipline. Has somatic worries and difficulty in harnessing his impulses. Shows dependent relationship with the mother figure and hostility toward women. Had problems with a suitor of one of his sisters. Is reserved and cautious. Sexually inadequate and unbalanced, he feels a need for virile assertion compensatory for homosexual trends. His patron god, Yansan, is fiery, benevolent; Ogun is severe when necessary; Orishala is lenient but punitive. Experiences possession. Joined the cult 5 years ago because of emotional problems and physical illness. His sister is an initiate. He first attended a spiritualist session.

Case 17. Age 21; extroverted, immature, egocentric. Has difficulty with social relationships, feelings of deficiency and debility. Shows compensatory needs for power and dominance, indecision, evasiveness in contacts with environment. Has weak intellectual capacity. Is dependent on and strongly attached to the mother figure. Shows hostility toward women and debasement with feelings of dominance. His sexual problems are sexual inadequacy, ambivalence toward sex, and homosexual trends. He is also ambivalent toward his patron god, Shango; Yemanja is "better." He was raised at a cult house as an adopted child of the priestess. Complains of cephalalgy. Never experienced possession.

Case 23. Age 25; psychosexually immature, dependent, narcissistic, with needs for power and social dominance. Is timid, insecure, evasive, depressive in social contacts. Was evasive and defensive in the interview. Shows feelings of inferiority, with compensatory aggression and arrogance. Started working in agriculture at age 14. Shows aggressiveness in relationship with the feminine figure. His mother, with whom he lives, tried to put an end to his vagrancy. He was unemployed at the time of the interview. His sexual problems are his concern about autoerotic practices (guilt), need for virile assertion, effeminacy, awareness of sexual debility, homosexual trends. Shows conscious and apprehensive tension. His patron god, Shango, is violent; Yansan is "bad-tempered." His mother is a leader at a spiritualist center. Joined the cult 5 years ago because of physical illness, difficulty in finding a job, quarrels. Experiences possession.

Case 25. Age 25; introverted, egocentric, narcissistic, infantile, with difficulty in social relationships. Despises people, is sensitive to interpersonal relationships, and needs to appear socially dominant. Has exhibitionistic tendencies. Values social dominance; is ambitious, insecure, aggressive toward his environment. Attributes dominance and power to women. Is mother-dependent and psychosexually immature. Shows hostility toward women, with difficulty in the relationship with the feminine figure. His sexual problems are conflict in heterosexual relationships, guilt feeling over masturbation, effeminacy, homosexual trends. Is reticent about his gods. Joined the cult 8 years ago because of physical illness. Experiences possession. Unemployed.

Case 47. Age 52; extroverted, egocentric, infantile, with feelings of inferiority. His contact with the environment is aggressive, with guilt feelings. Is timid and insecure and dependent on the mother figure. Never had steady love relationships. Shows difficult relationship with the feminine figure. Became engaged at age 20, but his fiancée, after ten years of waiting, broke the relationship and fell in love with another man. He married when 36 years old. Shows conflict over sexual inadequacy and homosexual trends. Is reticent about his patron god, Ogun. Joined the cult because of a love problem. Never had possession.

Case 50. Age 59; extroverted, narcissistic, egocentric. Has difficulty in environmental relationship, with repressed aggressiveness, need for dominance. Is compulsive, with exaggerated tendency to order. Shows superficial sociability. Says he likes carnival and St. John's eve celebrations. "I have a mad desire to spend my money and light fireworks for St. John." Is dependent on the mother figure. Married when 35 years old. His sexual problems are: conflict with sex involution, deviant sexual behavior, lack of adaptation, homosexual trends. Is reticent about his patron god, Oshala; Shango is punitive; Yemanja is compassionate; Oshun is wealthy. Joined the cult 10 years ago because of physical illness. Retired from his job because of heart ailment. Experiences possession.

Case 51. Age 31; introverted, egocentric, narcissistic, immature. In social relationship, reveals a great degree of insecurity, timidity, depression. Is superficial in contacts, evasive, with traits of ambition, exhibitionism, ostentation. Makes prolix statements like: "I say Christian because those who live at the Church and know how to fulfill Christ's designs all are Christians." Or "according to my psychology," etc. His relationships with the mother and feminine figures is hostile and aggressive. Is ambivalent, inadequate, and conflictive in relation to sex. Strives for virility in compensation for his insecurity in masculine affirmations. Is reticent about his patron god, Ogun, and his other god, Ode; calls Yemanje peaceful, Oshun "passive," gay. Also has an Indian spirit, who is strong and punitive. Joined the cult because of familiarity (his whole family belongs to the cult; a brother is a leader at another house). Experiences possession. Claims to have "visions."

Case 56. Age 62; regressive, hostile, psychosexually infantile, and narcissistic. Has difficulty with social relationships; is hostile to the environment, distrustful and aggressive in social contacts with tendency toward exhibitionism. Is ambitious. Lacks stability; has a feeling of physical inadequacy and needs to look physically strong. Shows hostility toward the feminine figure and difficulty in relating to it. Is reticent about sexual matters: shows sexual inadequacy and confusion, with homosexual trends. Displays traits of schizophrenia. Is reticent about his gods. Joined the cult 8 years ago because of physical illness. Experiences possession.

Case 57. Age 18; introverted, narcissistic, egocentric, immature, with deep psychosexual insecurity. Shows difficulty in social relationships, with need for power and domination. Values the intellect. Is sensitive, evasive, reacts aggressively while in contact with the environ-

> ment. Tends toward exhibitionism; is ambitious. Is dependent in his relationship with his mother. Shows attachment to and identification with the mother figure. Has difficult relationships with the female figure. Shows conflict over virility, deviant sexual behavior, oral eroticism, sexual immorality. Is reticent about his gods. Joined the cult two years ago because of schizophrenic symptoms, suicidal ideas. Never had possession. Is ashamed when dancing at the ceremonies.

Almost all the subjects whose cases have been summarized here felt sexually inadequate because of doubt about their virility, or homosexual tendencies, or their hostility toward the female figure. Some even resorted to autoerotic practices and sexual immorality. They are attached in various degrees to and dependent on their mothers and also had different kinds of difficulties with their social relationships. Either timid, suspicious, or insecure, depressed, and aggressive, most of them have developed needs for social dominance and assertion or have showed open aggressiveness with guilt feelings over it. Psychosexually immature, narcissistic, some of them effeminate or introverted, egocentric or infantile, they are ambivalent or reticent about the figures of their gods, only in a few cases projecting upon them the figures of the familiar constellation. Half of these subjects never experienced possession, which indicates deep emotional disturbance, as Melville J. Herskovits pointed out some time ago (Herskovits, 1936).

It should be said, furthermore, that irrespective of traditionalism and syncretism these sexually inadequate or ambivalent individuals, as would be expected, were found in most cases (five out of nine) at houses led by homosexual priests, like the overt homosexuals analyzed at the beginning of this paper.

GENERAL COMMENTS

The subjects whose cases we have analyzed had very little education: 29 percent were illiterate; 21 percent had rudimentary schooling; 41 percent had attended grammar school; 9 percent had gone to high school. Their education was commonly interrupted between the ages of twelve and fourteen years because of their need to have a job earlier than adolescents in other social classes. Most of them are common laborers (24 percent), or artisans (26 percent), a few reached the lower-middle-class occupations (15 percent), while 9 percent were domestic servants and another 9 percent were unemployed.

Hostility toward the environment, aggression, needs for power and dominance in their social relationships, or other difficulties like insecurity, timidity, anxiety, suspicion, and so forth, are not surprisingly found among these subjects. Their social conditions of lower-class, deprived people will undoubtedly account for much of the psychological stresses revealed by the tests and the interviews, together with the consequent introversive, insecure, sensitive, aggressive, and guilt-laden personalities thereby developed. Family constellations and family life conditions often detrimental to a harmonious and well-balanced personality growth influence from early infancy the child's attempts at establishing a rewarding system of relationships with parents, parent surrogates, siblings, and other figures in their environment.

African cultural patterns and beliefs, retained or reinterpreted, may have influenced the enculturation and socialization of our subjects through handy mythical models for behavior, role-determining ascriptions, tension-releasing outlets, and specific patterns of child care and family life. However, the small number of identifications of our subjects with their patron gods (5 out of 34 cases) and of projections of family figures on those and other gods are noticeable. Five subjects with authoritarian father images also had severe patron gods; but three others with similar father images had lenient patron gods, and another had an authoritarian patron deity. The same applies to those with authoritarian mother images. Only three also had authoritarian patron gods; two, authoritarian patron deities; two others had ambivalent images of their patron gods, and one had a lenient patron deity image. It is possible that the projective mechanisms seen to operate in the cases of possession studied through the sequential Rorschachs as reported elsewhere (Ribeiro, 1959) would not have been relied upon by most of our subjects.

Without going beyond our interview and test evidence, we may recall that health and economic needs were the main reasons for our subjects' having joined the cult (21 percent and 11 percent respectively). Familiarity, through indoctrination by parents and relatives already members of the cult, through habitual attendance at cult ceremonies, or through urging by spouse, fiancée, lover, accounts for only 20 percent of the membership. Furthermore, it should be emphasized that only members of the traditional groups showed a reasonable knowledge of mythology. Priests and priestesses refrain from passing on their esoteric knowledge, according to the best African tradition and also for fear of competition.

It would be surprising if people so low on the social and economic ladders entertained speculative preoccupation with theology or mythology. Rather they would seek relief from their sufferings, often from various sources simultaneously, at Afro-Brazilian cults, spiritualist sessions, and through scientific and folk medicine. They might even go to extremes, like our case No. 56, an attendant at a public psychiatric hospital, who participates in its therapeutic community and goes regularly to the Catholic masses held there, takes the medicines prescribed by the doctors for his heart ailment, and seeks relief at an Afro-Brazilian cult for the psychosomatic symptoms associated with his heart condition.

Both traditional and syncretic groups have among their members psychosexually disturbed personalities with varied psychodynamic complexes of determinants. It was possible in this study to build a typology of family constellations possibly correlated to the distinct classes of cult groups (traditional and syncretic) and linked to our subjects' ambivalence and difficulties with sex identification. These types also seem to be independent of the leadership of these groups (male, female, and homosexual). However, the small number of cases indicates that the assessment of a true correlation should be left for a more advanced stage of the research. Nevertheless, we cannot refrain from calling attention to the occurrence, at a syncretic house led by a priestess, of an exaggerated number of cases (five out of ten) of subjects with twisted sex identification. The personality type of the priestess as assessed through the D-A-P test shows a mother-centered, father-rejecting person with conflictive sexuality, insecurity, feelings of inferiority, also introverted, regressive, thus congenial to those subjects' personalities and close to their mother images.

Clarification of the operation of socioeconomic and cultural factors in modeling the personality of the lower-class subjects should, in our opinion, await the extension of the research to women and the results of the examination of a sample of the population not directly affiliated with the Afro-Brazilian cults. In our clinical experience, a number of patients from the upper-middle and upper classes have developed impaired sex identification and passive homosexuality from the injurious influence of family constellations like those of the typology presented above. Authoritarian fathers and submissive but permissive and protective mothers or energetic, punitive but possessive mothers and absent, neutral, submissive fathers, and their resulting introversive, narcissistic, egocentric, sensitive, aggressive, mother-centered, guilt-laden offspring striving for socal recognition and virile affirmation and also wrestling with their homosexual trends and their sex ambivalence and confusion will be found beyond the social layers infused with elements of African cultures. It would seem proper to recall here that Dr. T. Adeoye Lambo found no homosexualism in Nigeria or any prohibition there against it.[1]

Homosexuals were attracted to those groups led by homosexuals; alcoholics were found at two houses where controls are loose—one because of the homosexuality of the priest, the other because of its permissiveness in matters of orthodoxy and behavior. Nevertheless, one alchoholic was affiliated with a group led by a priestess who was a noteworthy example of austerity and ability as a leader. Subjects with a slight degree of sexual inadequacy and homosexual trends are scattered either among traditional groups (one in a group led by a male, two in another led by a female) or in syncretic groups (two in a cult led by a female, three in another led by a homosexual). This has little significance and may be merely a matter of chance.

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[1] Personal communication to Irving Bieber, reiterated to the present author while at this symposium.

Homosexualism is culturally defined. Tolerance toward what may be called deviant sexual or autoerotic practices, such as masturbation or stimulation of an infant's genitalia or sexual experiments between adolescents, assumes a great latitude according to the culture considered. Transvestites may not necessarily be overt homosexuals, and even they may be so considered only when "socially they take over the role of the other sex." (Kluckhohn, 1956). Peoples like the Trukese, the Carolinians, the Blood, the Ulitian, the Bisonhorn Maria, the Ute, the Vyane, the Djukas, and the Nigerians do not practice homosexualism, while the Siwans and Keraki and the Marind-Anim have institutionalized it. However, as pointed out by Marvin K. Opler, with the exception of ancient Greece, Japan before the Meiji dynasty, and some of the politically dominant German Nazi groups, or the Nata slaves, the Chukchee shamans and the *berdaches* among the Plains Indians, no society has ever approved homosexuality (Opler, 1967).

Two-thirds of the subjects in this study were intolerant of pederasty, with the exception of overt homosexual, alcoholics, and those affiliated with groups led by homosexual priests. They seemed to follow, at least when interviewed, the strict rules of Brazilian culture, which ostracize homosexuals, saying of them: "nauseous, the worst men on earth"; or "shameful," or "if it depended on me, there would not be any in the cult," etc. Some subjects were much concerned with the numbers of homosexuals at the cult houses, for fear that others would pass judgment on them, or that the cult would thereby lose prestige. When ambivalent, these followers adhere to naive ideas about biological determinism, such as "it is a vice of the flesh; therefore they have to comply with it." Other current ideas hold that homosexuals "have had the predisposition from infancy"; "they learn from others"; "female gods are the ones that choose them," etc.

Oshun, the deity with a mythical reputation for being lax, however, was found to be the patron deity of only one of the homosexuals and of three of the individuals with difficulty concerning sex identity. It was the secondary deity of the other homosexuals (as would be expected from tradition), but only 9 out of 25 subjects with difficulty about sex identification or sexual inadequacy and homosexual trends had this deity as one of their secondary gods.

CONCLUSION

From the evidence reported above, it may be said that the affiliation of homosexuals or people who have sexual problems with African-derived religions in Recife, and their attendance at the rituals, cannot be held responsible for their sexual deviations. The behavior of our subjects seems to stem rather from the complex of parent-child and other primary group relationships that shaped their early sexuality and tension-releasing mechanisms.

Homosexuals or people with impaired sexual adjustment may approach the Afro-Brazilian cult group for a number of reasons, among them the company of women, the need to display their mannerisms or to identify with female deities in congeries led by homosexuals or by austere men or women equally dedicated to the control of the supernatural. They also may be pushed by other complex motivational needs of compensation for the frustrations imposed upon them by their positions and interactions within the society at large.

Some of their behavior was rationalized through catchwords and simple formulas, or even by telelogical explanations. However, it remains a truism that human motives transcend the limits of simple and fortuitous explanation.

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DISCUSSION

Dr. Garn inquired about the mechanism of increased intelligence in the adrenogenital syndrome. Does it come about simply through increased rates of growth, or through a specific masculinizing hormonal effect on intellective acuity?

It is not related to masculinization after birth, Dr. Money replied, because those who have been kept well controlled from birth onward show, as a group, the same statistic in distribution of IQ. One cannot now decide whether it is some genetically selective factor that appears in carriers who have survived to produce a family with affected individuals in it. He and his associates had had a limited opportunity to test the brothers and sisters of a few of the children and had found that they tended to be of high IQ too. Another study of the siblings of these children is needed to find out whether there is a genetic factor involved. The most promising hypothesis at present is that the fetal androgenizing that changes the external morphology perhaps does something beneficial to the cerebral cortex. That has not been proved, however.

Dr. Martini called attention to the finding that masculinization of the human fetus can be brought about by giving progesterone to the mother. In animals, it is possible to protect the fetus from androgenization by giving progesterone simultaneously with testosterone during pregnancy. This is quite a surprising finding, and it has been confirmed. It has been found by Dr. Martini and his associates and also by Kinsell in New York and by Dorfman.

The finding is a paradox and hard to understand, Dr. Money said. It is also a paradox that progestin, which masculinizes the fetus, is an antiandrogen in adult life. He and his group have had some preliminary dramatic success in using Provera® (Upjohn), a synthetic progestin, to reduce androgen levels to near zero in male sex offenders, thereby making it easier to approach them in psychotherapy. The effect is time-related, not permanent. Progestin suppression of androgen is much better than the old way of using estrogen, because the patients do not get any breast development from it.

"It is quite evident from recent data," said Dr. Martini, "that the cerebral cortex is able to metabolize several steroids. I wonder whether experiments have been or should be done to see whether the administration of hormones during fetal life might increase or modify the ability of the cerebral cortex to metabolize the same or other hormones." Dr. Money thought it would be a good field for somebody to get into.

With respect to the dimorphisms at various stages that Dr. Money had mentioned, and the environmental influences on them, Dr. Hirschhorn said, there is a system that has polymorphism: the immunological system, which is really a counterpart to virtually the entire sequence of events described, such as intrauterine choices in the development of fetal tolerance and the postnatal influence on the selection for rapid mitosis of certain portions of the immunological system. The final product then developed has, as it were, an immunological identity as a counterpart to the gender identity. Dr. Money observed that the idea was indeed interesting to think about.

In response to Dr. Lambo's request that Dr. Money elaborate somewhat on his remark about tomboyism of androgenized girls, Dr. Money said that the most impressive finding was the heightened incidence of athletic interest and skills in these girls with the androgenital and progestin-induced forms of hermaphroditism in comparison with the matched normal control group. This difference in behavior was statistically significant, and the mothers of some of the girls were much impressed by it. Furthermore, there was a difference in the rehearsal of maternalism as seen in doll play, in play about pregnancy and childbirth. Most of these androgenized girls were not strictly against having children; they just were not concerned with it. If it was to happen one day, that was all right, but they really did not think about it as did those in the normal control group. With regard to clothing, which also was a clear-cut statistically significant difference, the normal control girls were much more interested in traditionally feminine dress than were the androgenized girls, who preferred casual slacks and shirts most of the time.

Dr. Richardson asked whether he had been correct in understanding Dr. Money to say that when a sex change has been accomplished, there is apparently complete satisfaction on the part of the person involved, with no regret later about the possibility of the decision having been incorrect. Dr. Money verified the statement, saying it was almost unheard of for a person to regret the change. "It seems as if this dichotomy has to have a weight that pulls one end of the see-saw down."

Sex reassignment, Dr. Money continued, whether for hermaphrodites or for the morphologically normal transexuals, changes only the legality of their gender role and their appearance and status in society. It does not change any possible pre-existing mental state, such as depression or paranoia. If the person has just an *idée fixe,* without any other psychopathological symptoms, the change does something that is very helpful to him (or her).

Dr. Gutmann said that some research he had been doing with older men in populations in different parts of the world had begun to suggest to him that within the normal life cycle, even before the gross breakdowns of senility, there is a beginning or emergence of what in some rough ways may be considered feminine interests. One finds older men become more nurturing, more maternal in their concerns. They become interested in young children, in growing things. They are somewhat fearful of competition and aggressiveness. They dislike such things in themselves as well as in the world. There is a tendency to ascribe strength to the outer world and a kind of weakness, passivity, and placidity to themselves. They take what is perhaps inaccurately considered a feminine stance toward powerful authorities, whether those authorities are culturally defined as gods or are some occupying power. The change shows up strongly in projective techniques in which one reaches some preconscious concerns. Dr. Gutmann wondered whether this change is in some way related to the bipotentiality that Dr. Money had talked about.

"I am amazed," Dr. Money replied, "that in psychological theories in general we take it for granted, in connection with aging processes, that people are going to stay the same throughout the adult years. I think they obviously do not. You have made a good point."

In reply to a question from Dr. Rossi about the timing of surgery in the correction of hermaphroditic children, Dr. Money said there are two answers. They have to do with the kind of operation involved, the kind of case to be corrected: whether sex reassignment or reannouncement is anticipated, or a surgical correction is to be made in the sex the child is already living in. In both cases, the first answer is: the earlier the better. It takes a burden of ambiguity away from the parents to have sex very clear in their eyes. It takes away the burden of memory from the child since he or she — it is especially "she" in this case, because you can do earlier, more complete surgery for feminization — does not have to remember the time when she had a "thing" down there. The delay that has been occasioned in many cases in the past, especially in the adrenal syndrome (because of the child's tendency to be a salt loser, which is increased under stress), has produced a bad psychological result. Early surgery can be well taken care of now with the proper medication.

As for sex reannouncement, Dr. Money continued, by the time a child of average intelligence is about eighteen months old, he or she already has pretty good use of the pronouns and proper nouns relating to sex. The child is saying, "I am a boy," or "I am a girl." A child is reminded of being a boy or a girl an astonishing number of times in a day. By that age there is a backlog of memory that has to be obliterated if a sex reassignment is made then. It is difficult for the parents and may be psychologically perilous for the child to reassign sex even at the age of only sixteen months. After ages sixteen to eighteen months, in such a case as a hermaphrodite with no penis at all and erroneously assigned as a boy because someone felt the testicles in the groin, it might be possible to effect the change, but only with good support from psychological or psychiatric follow-up. It is actually easier to do a reassignment with a much older child who has developed an ambiguous gender identity and who is able to go along with the doctor, to talk to him and collaborate with him.

In cases of feminization, where the operation has been delayed because the clitoris can be better dealt with surgically later, Dr. Money was not sure how valuable it is to try to preserve part of it. Is it difficult technically, so one is likely to end up without any of the organ saved. There are complications involved in trying to implant an enlarged clitoris under the skin. It is possible to destroy an extensive amount of the genital tissue without destroying the capacity for erotic response and orgasm.

Dr. Minturn, noting that in some societies, chiefly in Africa, a clitoridectomy is called a female circumcision, asked Dr. Money if there is any research indicating whether there is a correlation between that operation and genetic abnormality in relation to homosexuality.

Dr. Money thought that there is probably no specific connection between homosexuality and clitoral enlargement, but he said one should make a few cautious provisos. One is that in a genetically abnormal condition like the XXY syndrome, there is probably more homosexuality in the same way that there is more psychopathology of every type than in a randomly-selected population.

"From some of the newest material," he said, "it appears that there is more homosexuality in the XYY syndrome, too, although we shall need a bigger sample to be sure about that. It is not because the genetic defect causes homosexuality in a point-to-point correlation; the only sensible thing I can say is that it is a state of vulnerability in which psychosexual differentiation is somehow interfered with and impeded in a way that I do not think we can spell out yet. A very small proportion of the total population is XXY or XYY genetically. As far as the etiology of homosexuality is concerned in general, it is much safer for us to say we do not know than to get involved in a lot of fancy theories."

Dr. Lambo asked Dr. Money whether, if the genetic abnormality of the XXY chromosome leads to homosexuality in some cultures, the same thing may happen in other countries where there is still not any behavioral manifestation of homosexuality. Does it mean that, because of the assigned role from the child's birth, he will not later show any homosexual behavioral manifestation? Dr. Money's reply was that probably in a society that has a clear directive to children as far as psychosexual differentiation is concerned, the boy born with Klinefelter's (XXY) syndrome, if he is going to have any kind of personality instability develop, will show some anomaly other than a sexual one.

Dr. Money noted that in the United States there is a noticeable tendency for homosexuals to gravitate toward certain professions, especially the theatre. As for the theatre, Dr. Money's interpretation was that these people are by natural inclination good actors and role players. There may be a basic trait here that makes certain little boys more vulnerable to the development of a gender identity disorder in response to the special stresses and strains of life. Others may turn out to be juvenile delinquents in response to the same stresses. Dr. Money asked whether it might not be possible that some boys develop both their effeminacy and their acting intrests because of a basic talent for role playing.

The involvement of homosexuals with the theatre and their capacity for role playing suggested to Dr. Gutmann that one might think differently about gender confusion, at least as it applies to homosexuality. Persons who have made final decisions about sex identity think of those who cross the boundaries or maintain footholds on both side of them as being in a state of indecision. It may be, on the other hand, that their crossing of the boundaries in itself represents a kind of decision. Perhaps what is underlying homosexuality may not be sexual confusion, but rather the narcissistic attitude, the omnipotence strivings, which seem to say, "I will not make any final decisions. I will not be limited by what I consider arbitrary distinctions between masculine and feminine, restrictions that are imposed on me by cultural definitions. I will maintain all the gains. I will be both man and woman as I choose." Perhaps this narcissistic, omnipotent attitude in many homosexuals comes out in the descriptions of cult members as well.

Dr. Ribeiro remarked that there is definite evidence that the relation between the theatrical and dancing professions and homosexuality is correlated with the concern of these people with their bodies. These activities provide opportunity for displaying their mannerisms and for cultivation of more refined forms of physical expression.

Homosexuality, at least in many cases, Dr. Gutmann added, involves a route toward power. There is a strange kind of addiction to strength, a matter that is often left out in the consideration of the subject. In the homosexual act one literally stoops to conquer. The inner fantasy is that one attracts the power of one's partner through homosexual submission to him. In many cultures, in the shaman role, too, the homosexual literally dresses like a woman, is overwhelmed by the gods, suddenly is filled with power, and announces himself as the god. In some strange way, this feminine motif is a route to power.

This may also have something to do with the frequency of alcoholism one finds, both among homosexuals and in the cults that Dr. Ribeiro has studied, Dr. Gutmann continued. The alcoholic's relation to alcohol in many cases is much like the homosexual's relation to his heterosexual or "masculine" partner. Again, as many alcoholics see it in their inner fantasies, alcohol is a strong substance, which first overwhelms you, but if you can overcome it, if you can integrate with it, then you are filled with strength; you experience a kind of omnipotence.

Dr. Minturn suggested that the choice of occupation for a homosexual may be a consequence rather than an antecedent. One may go into an occupation in which he knows he will find other homosexuals. She thought also that there may be a tendency not only to enter feminine areas, but also to sabotage them to some degree, as appears to be the case in design of women's clothes and interior decoration.

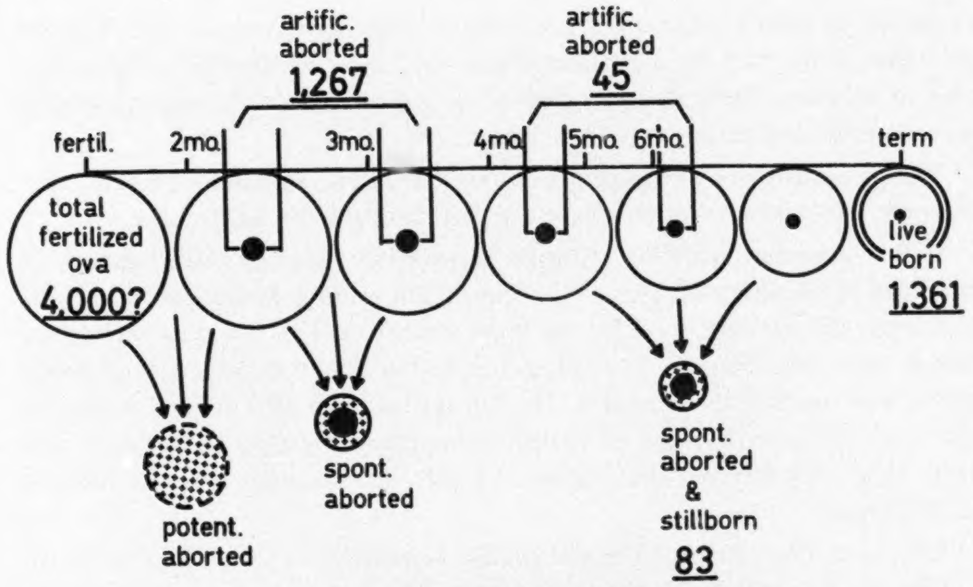
The possibility of predicting homosexuality in children interested Dr. Barnett. He asked whether there are any data on the subject.

It is a question without a simple answer, Dr. Money said, because in the kind of homosexual play he had observed among Australian aborigine children, the activity qualifies as homosexual on the behavior criterion, but it does not become part of a subjective inner experience of erotic attraction toward the same sex. He thought one should allow for exactly the same thing with much of childhood exploratory play in our own culture. It all depends on the degree of inner involvement and the motivational factor.

The whole question of homosexuality is something we should be cautious about, Dr. Money continued, because it includes different personality types and different styles of behavior. In some prisons, for example, some of the men are nicknamed gorillas. They test new inmates for timidity. A small man who is likely to yield to a threat and is intimidated by fear of reprisals if he tells is raped and is used homosexually in sodomy throughout his prison term, unless he is somehow rescued by the administration. The gorillas do not continue to be homosexual when they leave prison, but in prison they qualify as homosexuals on a behavioral criterion.

Dr. Money added that he thought there was not yet enough evidence from longitudinal studies to enable anyone to make confident predictions about the child who shows an effeminate type of gender identity. In twenty youngsters he has been following he has found that there is a good deal of continuing gender confusion as they grow up. Some of them unquestionably will graduate into an ordinary adolescent career of homosexuality. Some of them may undergo a variety of changes in psychopathology. At age eighteen it may be that no homosexuality will be evident. In such individuals, Dr. Money suspected that there will not be much sexuality evident at all, along with other personality impairments.

Dr. Nishimura commented on, and illustrated, the incidence of hermaphroditism. It is said to be 0.2 to 0.3 percent in the postnatal population (Overzier, C. *Intersexuality*, London, Academic Press, 1963). But the study by S. Lee of his group based on a large number of embryonic population born of mothers with healthy courses of pregnancy showed that of the embryos examined at about six to seven weeks of estimated fertilization age, the incidence was about one percent—several times higher than the figure on the postnatal population (Table I). A diagram shown as Figure 1 gave the background of Dr. Nishimura's explanation. His studies of embryos in the second to fourth month of pregnancy show that the


artific.
aborted
1,267
artific.
aborted
45
fertil.
2mo.
3mo.
4mo.
5mo.
6mo.
term
total
fertilized
ova
4,000?
live
born
1,361
potent.
aborted
spont.
aborted
spont.
aborted
&
stillborn
83

All figures are shown in thousands.

●: Their various sizes represent comparative value of prevalence of malformed embryos or fetuses among various populations.

FIGURE 1.–Diagram showing the fate of fertilized ova of total Japanese population in 1966 (ca. 99 million).

population at that stage of organogenesis is different from that of the postnatal population (Nishimura, H. *Okajimas Folia Anat. Jap.*, 46, No. 6, 1946, in press).

A question from Dr Money about Dr. Nishimura's definitions of "lateral, unilateral, and bilateral hermaphroditism" in Table I brought the answer that the terms referred to the gonad of opposite sex on each side, the one- or two-sided ovotestes of the embryos. Therefore, Dr. Money noted, Dr. Nishimura was dealing only with true hermaphrodites, not with the adrenogenital syndrome or the hypospadiac male type of hermaphrodite. Dr. Nishimura said that was correct. In that case, Dr. Money observed, the incidence is quite high, because the live cases of true hermaphroditism as compared with pseudo-hermaphroditism are very rare.

TABLE I—Prevalence of true hermaphroditism detected by histology of gonads (Lee, S. & Nishimura, H., unpublished)

Embryos					Total population
Stage (CRLmm)	Total no.	Sex ratio	Hermaphrodites Type* and no.	Prevalence per 1000	Prevalence per 1000
13-30	1328	156	B 2 L 9 U 4 } 15	*11*	<2-3 (Overzier, 1963)

* B: bilateral; L: lateral; U: unilateral

CROSS-CULTURAL RESEARCH ON HUMAN BEHAVIOR
A COMPARATIVE STUDY OF THE LIFE CYCLE IN THE MIDDLE AND LATER YEARS

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The periods of middle and later life have not been particularly well comprehended by psychologists. We do not have many large conceptions to help us organize the findings from the great number of already completed studies on perception, cognition, attitudes, etc., in later life. By contrast, on the other end of the life span we do have a host of large, ornate, dynamic theories concerning psychological development in infancy, childhood, and adolescence; but the prevailing assumption concerning middle and later life is that true psychological development ends with physical maturation, and that the individual coasts through the rest of his life, acting out the traumas and acting on the capacities that are the residues of the early developmental years.

However, a few psychologists have begun to extend our conceptual grasp into the later reaches of the life cycle. Cumming and Henry (1961) have proposed that older people are intrinsically moved to disengage themselves from the normative systems of social control that ordered their behavior during the productive and responsible middle years; and Erik Erikson (1959) has sketched out a sequence of ego stages, extending across the total life span, that presumably demarcate the course of psychological development from infancy through middle and later life — that is, well beyond the periods of intense physiological and sexual development.

Along similar lines, I have been attempting to get at life-cycle, or "species," characteristics of the psychology of middle and later life, through the method of cross-cultural comparison. Thus, I have been making intergenerational comparisons across a panel of traditionally oriented, preliterate, Mexican Indian, American Indian and Middle Eastern groups (Gutmann, 1966, 1967). Preliminary findings suggest that certain psychological dimensions consistently differentiate younger (35-54) from older (55+) men across the panel of subject societies. The part of the intergenerational variation that is consistent across cultures cannot be accounted for by extrinsic or cultural influences, since these are different at each site. Accordingly, we begin to look for intrinsic organizers of these generational shifts, agents that are possibly developmental or genetic in nature.

These cross-cultural and intergenerational comparisons have been made in terms of a typology of "Ego Mastery" stages. These mastery orientations were empirically derived from analyses of TAT (Thematic Apperception Test) data provided by middle-class Kansas City men aged 40 through 70, where each mastery type refers to a distinctive integration of needs, ego defenses, and cognitive styles. In less technical terms, each mastery type refers to a particular way of relating to the self and to the world, to a particular way of understanding the world, and to a particular way of creating, defining, and solving problems.

These ego states are, of course, not mutually exclusive. The same person can display them all in different contexts or at different times within the same context. I present them here as exclusive states for heuristic purposes, to highlight real intertype differences. In addition, the typology does have an empirical basis. Men sorted into these types on the basis of TAT performance were also discriminated by interviewer's ratings of overt behavior and affect, by performance on the Draw-a-Person test, and by the Life Satisfaction rating—a validated scale devised by Bernice Neugarten and her associates (1961).

Before discussing the age X mastery type distributions in the various cultures, I will indicate the content of the major orientations and of their component subtypes.

The first major type I call Alloplastic or Active Mastery. This orientation seems to have as its motivational foundation strivings toward autonomy, competence, and control. The Active-Mastery person works within external action systems to achieve maximum effect on them, to bring them, or some part of them, under his control. Such individuals tend to require much independence of action and choice. Individuals of this type are wary of having their actions and choices limited by others, and they accordingly mistrust dependent yearnings within themselves that might lead them to trade compliance for security.

These various dispositions add up to what might be called an "active-productive" orientation. Like all men, the Active-Mastery person desires emotional and physical security, but he tries to gain them on his own; and he tries to be a source of security to others. He is, therefore, most comfortable with resources—a business, a flock, a cornfield—that he has created for himself and for his dependents through his own competence and effort.

There appear to be two subtypes of Active Mastery: the Promethean-competitive and the productive-autonomous. These may coexist within some Active-Mastery individuals; alternatively, they may differentiate between men of this general orientation.

The Promethean subtype emphasizes combat and competition. Strength and prestige are trophies won from an enemy. Whether the enemy is a person or a piece of refractory nature, the Promethean feels that he has fought for his assets and for his territories.

The autonomous-productive individual is self-reliant and hard-working. For him, there is less emphasis on external enemies and challenges and more emphasis on living up to high internal standards. He competes, in effect, against himself.

The second major type I call Passive or Autoplastic Mastery. Individuals of this orientation do not feel effective enough to create and control the sources of their own security. From their standpoint, strong and probably capricious agents control what is needed and valued. The Passive-Mastery individual can influence the powers-that-be only indirectly, through what he does to himself. Such people tend to reshape themselves to fit the expectations of the powerful and arbitrary providers. To this end, they exhibit mildness rather than challenge, and they try to eradicate aggressive tendencies that might lead them into dangerous conflict with the authorities. Accommodation and conformity to outer pressure are the chief attributes of this orientation.

Included within the overall Passive-Mastery framework are the anxious-constricted and emphasized-receptivity subtypes. Again, these may be found together within some Passive-Mastery persons, and they can differentiate between others. In the anxious-constricted case, passivity is the consequence of fearful inhibition and often hides covert aggression.

The chief attribute of emphasized-receptivity is nurturance: to and from the self. Receptive persons need affection and nutriment from others; they try to please potential donors by meeting their requirements. They are happiest in relationships that involve giving and getting emotional and physical support.

The third major type is Magical or Omniplastic Mastery. In this stage, the world or the self are revised projectively, by perceptual-cognitive *fiat*, not instrumentally. Regressive ego defenses replace both autoplastic and alloplastic instrumentality. Thus, dangerous or troubling events in the outer world are defined as innocuous or benign (denial); or others are held responsible for one's own troublesome thoughts or actions (externalization or projection).

TABLE I—Distribution of the Kansas City male sample by major age periods, and by mastery orientations

		40-49		50-59		60-71		
Active mastery	Promethean-Competitive	7*	17	8	26	3	12	
	Productive-Autonomous	10		18		9		
Passive mastery	Emphasized Receptivity	8	8	20	26	12	23	
	Anxious Constriction	0		6		11		
Magical mastery		4		15		14		
		29		67		49		145

* This distribution of the mastery cell sub-totals significant at the .02 level ($x^2 = 17.417$; DF = 8).

For the Kansas City sample, judgments as to respondent's mastery type were made — blind for the age variable — from TAT data. As shown in Table I, there was an association between age and mastery type, in the predicted direction, significant at the .02 level: younger men — those in the forties and early fifties—cluster in one or another of the Active-Mastery subtypes; Passive Mastery and (to a lesser degree) Magical Mastery characterize the sixty- to seventy-year-olds.

These findings had developmental implications: in later life sequence, active → passive → magical mastery seemed to replicate, though in reverse order, the staging of ego development in childhood and adolescence. Thus, during the early years, the normal progression is from animistic thinking (Magical Mastery) during infancy, through obedience and dependency in childhood, (Passive Mastery) and finally to self-initiated productive activity in late adolescence (Active Mastery). The developmental hypothesis that came out of the U.S. findings has been tested across a range of cultures that are in varying degrees generationally homogeneous, preliterate, and traditional in orientation. At the same time, these societies differ greatly from each other in terms of such cultural variables as value systems, child-rearing practices, and age-grading systems. These groups are the lowland and highland Maya of Mexico, the Western Navajo of Arizona, and the Druze tribesmen of Israel. (I am still doing field work with the latter group, and cannot as yet report any Druze findings.)

TAT and interview data were collected from representative men, mainly in the 35-75 age range, at these sites. The general prediction has been that the age distribution of mastery orientations would be replicated in societies that differ from each other and from the urban United States in terms of the variables that presumably influence personality development. Again, if specified mastery orientations are found to distribute more predictably by age than by culture, they can be seen as demarcating a psychological continuum that has developmental implication.

As in the Kansas City case, the approach to the cross-cultural TAT data has been exploratory and inductive. The basic analytic units have been the individual stories generated from one respondent by one TAT card. The goal of the analytic procedures has been to make themes and trends that are (1) implicit in data and (2) relevant to the ego-mastery conceptions, explicit and comparable. The strategy is to derive a basis, appropriate to the data and indigenous to the respondent's culture, for assessing the central, motivated tendencies of individual stories, so that accurate judgments can be made concerning the ego position — active, passive, or magical — communicated by story form and particularly by story content.

Thus far, the data analysis has concentrated on those cards that were used without modification at research sites. These include the "Rope Climber" and the "Heterosexual" cards of the standard Murray TAT set (used in Kansas City, Druze, and all Indian sites) and the "Desert Scene" (used only at Druze and Indian sites). The data developed by these cards best illustrate the findings that have been emerging from the analysis as a whole.

The "Rope Climber" card suggests to many respondents — in any culture — a vigorous, muscular, and possibly nude figure who could be going up or down a rope. Because the card suggests nudity and strength, and because it does not depict social agents, the stimulus might be regarded as a representation of the impulsive, "instinctual," aspects of life. Hence, the card issue is presumably "impulsive vigor" and the card presumably "asks" the respondent: "What is *your* conception of strength, of impulse, and where do you locate these qualities?" Accordingly, respondents' estimates of the qualities, goals, and activities of this figure were regarded as metaphors of their personal relationship to their own impulse life. The various thematic representations of this relationship were translated into mastery orientations, the result being a set of thematic categories that accommodated all the data, regardless of cultural origin, generated by this card.

Active-Mastery stories were those in which the rope climber was seen to be impelled by his own intrinsic energies into competition, danger, or productive activity. And if the climber is menaced or destroyed, it is by agents that he himself has confronted or challenged. In Passive-Mastery stories, the hero is menaced by dangers that he has not sought out; or he performs, without zest, in conformity with some convention that requires the climbing of a rope. In either case, the hero is mainly reactive to outer forces and initiatives. In Magical-Mastery stories, the hero's energy is completely denied (he is dead). Conversely, the dangerous forces of the environment are grossly magnified (the rope is on fire).

Grouped by their respective mastery orientations, the specific categories are:

Active Mastery

Promethean

1. *Challenge and Competition:* The hero demonstrates his strength, usually in successful competition. However, the rope may break at the moment of triumph; and the respondent himself may deride the hero as a "show-off."

Productive-Autonomous

2. *Productive Effort:* The hero strives vigorously, sometimes zestfully, towards a self-determined productive goal. He does not compete against others nor flaunt his strength.

Passive Mastery

Anxious Constriction

3. *Externalized Inhibition:* The hero is immobilized by environmental agents which do not collaborate with his action, or which block it: the rope is slack; the cliff is slippery.

4. *Threat from Internal or External Aggression:* The hero is threatened by destructive external forces; alternatively, the hero's aggression is turned against himself (suicide) or is out of control and constitutes a threat to others (the hero is homicidal).

Role Constriction

5. The hero climbs, though without much involvement, for conventional purposes. Or, the respondent conforms to his role as subject, by giving a minimal though accurate description of the card.

Syntonic Passivity

6. *Somaticized Passivity:* The hero lacks force to match his purpose: he is tired or ill.

7. *Sensual Receptivity:* The hero has hedonic or security-seeking (rather than productive) purposes: he plays on the rope; he dives into water; he climbs to see something, to get a morsel of food, or to find his home.

Magical Mastery

8. The hero is not erect; or, the rope is not a rope (the hero is lying down; the hero is wounded; the rope is a snake, etc.).

TABLE II—The "Rope Climber" card: distribution of stories by age, culture, and theme

		35-49		50-59		60+	
Active mastery							
A. *Promethean*	Kansas City	21		33		10	
1. Competitive	Trad. Navajo	5	31*	1	36	6	19
	Lowland Maya	3		2		2	
	Highland Maya	2		0		1	
B. *Autonomous*	Kansas City	4		10		7	
2. Productive	Trad. Navajo	6	15	6	19	10	20
	Lowland Maya	3		2		2	
	Highland Maya	2		1		1	
Passive mastery							
A. *Anxious constriction*	Kansas City	—		3		—	
3. External inhibition	Trad. Navajo	—	7†	1	4	—	0
	Lowland Maya	6		—		—	
	Highland Maya	1		—		—	
4. External aggression	Kansas City	3		7		12	
	Trad. Navajo	3	8	—	8	7	21
	Lowland Maya	2		1		1	
	Highland Maya	—		—		1	
5. Constriction	Kansas City	1		11		5	
	Trad. Navajo	1	8b	5	23	9	26
	Lowland Maya	1		5		7	
	Highland Maya	5		2		5	
B. *Syntonic passivity*	Kansas City	—		1		—	
6. Somatic passivity	Trad. Navajo	—	0†	1	2	2	2
	Lowland Maya	—		—		—	
	Highland Maya	—		—		—	
7. Sensual receptivity	Kansas City	2		2		7	
	Trad. Navajo	3	12	3	17	10	38
	Lowland Maya	3		5		14	
	Highland Maya	4		7		7	

TABLE II—The "Rope Climber" card: distribution of stories by age, culture, and theme—continued

Magical mastery								
	8. Magical mastery	Kansas City	—		2		2	
		Trad. Navajo	—	1	—	4	3	8
		Lowland Maya	—		1		2	
		Highland Maya	1		1		1	
			82		113		134	

Kansas City N = 144
Trad. Navajo N = 81
Lowland Maya N = 62
Highland Maya N = 42
Total N = 329

* Chi Square (of cell totals) = 42.558, DF = 12, P < .001

† These cell totals combined with those of next lower category for computational purposes.

Table II presents the distribution of responses: by the above card categories, by age, and by culture. Though societies vary in the over-all degree of activity and passivity that they ascribe to the rope climber, the between-age cohorts comparisons, within and across cultures, indicate that younger men to a statistically significant degree favor the more active, productive possibilities, while older men generally favor the more hedonic, inert, less instrumental possibilities. Most respondents, regardless of age or culture, continue to see the hero as moving upwards, but the meaning that they assign to this activity shifts fairly drastically with age. For younger men, the hero tends to move against human rivals or abrasive nature; but for older men, if the hero strives against an enemy, it is more likely to be some passive and vulnerable aspect of himself, (the hero exercises to avoid illness and laziness).[1] For the most part, the older respondents see the hero's activity as being without particular aim; ("I see that he's climbing; I don't know why") or as part of a more comfort-oriented search for food, shelter, amusement, or esthetic experience ("he's climbing to get a better view").

The "Heterosexual Conflict" card of the Murray TAT depicts a young man turned away from a young woman who reaches towards him. Like the "Rope Climber," this card has been shown to male respondents at all sites. In the typical drama, conflict is proposed between an angry, sometimes rejecting man and solicitous and/or retentive woman. Thus, the card issue concerns the contrast between potentially dangerous male energy and more nutritive, less intrusive "female" qualities.

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[1] Note in this regard that the autonomous-productive component of Active Mastery does not in all cases decline with age. Accordingly, men who can sublimate their aggressive energies towards autonomous, achievant stances may maintain the Active Mastery orientation well into later life. Thus, cultures and subcultures which stress the achievement motif, and which thereby offer individuals this sublimated channel for the expression of potentially combative sentiments, may conserve Active Mastery among a large proportion of the older male population.

Accordingly, Active-Mastery stories portray a vigorous young man, intrinsically impelled towards productive, amorous, or combative exploits, who is relatively impervious to either the tenderness or the fear expressed by the young woman. Passive-Mastery stories are those in which significant power is located outside of the young man: a domineering, retentive woman restricts the outward movement of a still assertive young man; or a young man, defeated in or menaced by the outer world, moves back to the comfort and security offered by the young woman. Magical-Mastery stories are those in which the usually noted conflict possibilities are overlooked and the emphasis is instead on some peaceful relationship between two relatively undifferentiated individuals.

Grouped under their respective mastery orientations, the specific categories that accommodated all card data, from all cultures thus far analyzed, are these:

Active Mastery

Promethean

1. *Male Aggressive Initiatives:* Young man's intrinsic sex aggression and autonomy demands constitute a problem for a gentle, nurturant young woman and potential danger for himself.

Autonomous

2. *Male Autonomy Needs:* Direction or outcome of young man's aggression is usually nuclear, but seems mainly to involve rejection of the young woman's nurturance.

Passive Mastery

Externalized Inhibition and Aggression

3. *Female Initiatives and Dominance:* Young man's anger is reactive to young woman's rejection of him or dominance over him.

Syntonic Passivity

4. *Rationalized Male Succorance:* Menaced by external forces, or defeated in his outer-world achievement strivings, the young man looks for or accepts female nurturance and control.

Magical Mastery

5. *Untroubled Affiliation (or Syntonic Dependency):* Mild, untroubled affiliation between relatively undifferentiated young man and woman.

TABLE III—The "Heterosexual" card: distribution of stories by age, culture, and theme

		35-49		50-59		60+	
Active mastery							
A. *Promethean*	Kansas City	21		12		10	
1. Male aggressive initiative	Navajo	9	36*	7	20	9	21
	Lowland Maya	4		1		2	
	Highland Maya	2		—		—	
B. *Autonomous*							
2. Male autonomy needs	Kansas City	1		3		—	
	Navajo	4	12	4	14	4	12
	Lowland Maya	7		5		7	
	Highland Maya	—		2		1	
Passive mastery							
A. *Externalized aggression*	Kansas City	6		5		6	
3. Female dominance	Navajo	—	9	3	9	9	19
	Lowland Maya	—		—		2	
	Highland Maya	3		1		2	
B. *Syntonic passivity*							
4. Rationalized male succorance	Kansas City	—		1		4	
	Navajo	—	1	1	3	12	20
	Lowland Maya	—		1		2	
	Highland Maya	1		—		2	
Magical mastery							
5. Untroubled affiliation	Kansas City	—		—		—	
	Navajo	1	12	6	16	6	26
	Lowland Maya	3		7		12	
	Highland Maya	8		3		3	
	N =	70		62		98	

Kansas City N = 69
Navajo N = 75
Lowland Maya N = 53
Highland Maya N = 33

* Chi Square (of cell totals) = 24.643, DF = 8, P< .005

Table III indicates the age × theme × culture distributions to this card. Again, the between age cohorts across cultures comparisons tend to conform to predictions: a later life shift towards the passive and magical end of the psychological spectrum was observed in the data from three out of the four societies studied. Along these lines, Table V shows that in all but the Highland Maya case,[2] Active Mastery declines steadily across age groups, Passive Mastery rises steadily across age groups, and if Magical Mastery appears at all, it is always higher among the 60-year-olds than among the 40-year-olds.

In the main, then, the intergenerational, intercultural comparisons in regard to the "Heterosexual" card tend to replicate the results from equivalent "rope climber" comparisons; these provide further evidence of the shift away from exuberant and outward-directed male aggression towards more security-seeking, receptive stances. Thus, younger men propose that the male hero brushes aside a beseeching woman and pushes out into a dangerous but also exciting world of combat, carouse, and mistressses; while to the same stimulus older men propose themes of caution and depletion. In their version, the young woman tends to domineer; or the male protagonist retreats back to her consolation and away from a world in which he has known danger and defeat. In either case, initiatives and strength have migrated away from the young man towards the young woman or towards vaguely defined external agents that threaten the young man or the young couple. Finally, for many older men, the male protagonist does not reject the nurturance offered by the young woman, but instead dwells with her in happy, seamless harmony. Potential trouble comes from outside, not within, the dyad, and menaces the young man and woman equally.

In sum, two cards that raise for respondents the issue of the nature and deployment of masculine energies agree on the outlines of an age-graded transcultural consensus around these matters. That is, young men define strength as an ambivalently valued internal resource. The strong, competitive man also stirs up trouble for himself (the rope climber can fall at the moment of triumph; the young man rejects the comfort of a woman for the impersonal nurturance of a bottle and gets into drunken fights). The old men also retain this ambivalent relationship towards strength — it can help them or hurt them — but they see it to be lodged outside of themselves, in relatively capricious agencies, institutions, or authorities. Where the young men have to control their own aggression (because it can get them into trouble or cause hurt to their dependents), the older men have to control and deflect external aggression that, as they see it, potentially targets on them. Through their display of mildness and benevolence, they perhaps mime the good treatment that they hope to get from powerful secular and/or sacred authorities.

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[2] Both the younger and older Highland Maya men depicted the young man figure as mild and inert relative to the outer world or the young woman figure. But men of this group are very inhibited and fearful concerning sexual matters, and their anxiety may have been reflected in their particularly constricted definitions of the young man figure. Thus, the data generated by this card from this group may not be comparable to data from the other societies where sex is less problematic.

Both cards also agree on the age shift to more openly needful and incorporative positions, towards immediate affectional and sensual supplies.[3] In their apparent need to turn the world into a secure storehouse, older men will determinedly overlook potential conflicts and troubles that are clear to younger men. Thus, the older men, as predicted, rely on defensive denial rather than on action to remove the sense of threat. Instead of "taking arms against a sea of troubles," they may revise their perception and claim that the world holds neither troubles nor arms.

The predicted later life swing towards denial, the ego defense that is pivotal to the stage of Magical Mastery, is best illustrated through the data generated by the "Desert Scene" TAT card. This depicts a barren landscape, gullied in the foreground, dotted with cow skulls, empty of people, but transected by a trail and by barbed wire fences.

The majority of Indian respondents (both Mexican and American) visualized in this card one or another version of aridity, which was judged to be a response to the implicit issue of "innurturant environment." Category criteria took account of the degree to which respondents recognized this aridity, proposed reasonable human efforts to counteract it, or tried to deny it. Thus, Active-Mastery categories grouped those stories in which the reality of a hostile environment was admitted, without palliation; and also those stories that proposed restitutive human effort. Passive-Mastery categories grouped those stories in which it was proposed that restorative rain or water would appear through some natural agency, not under human control. Magical-Mastery categories grouped those stories in which key elements of the scene were misperceived, and it was proposed that water already covered the ground, or that helpful supernatural agents were concretely present on the scene. The particular categories grouped by mastery orientations are as follows:

Active Mastery

Coping (Promethean)

1. *Human Agency:* Emphasis on human effort in the face of a hostile environment; or on human artifacts (roads, fences, fields) that oppose the ravages of nature.

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[3] Older men seem to be more interested than younger men in all forms of sensual intake, not only through mouth and skin, but also through the eyes. Thus, for both the "Rope Climber" and "Heterosexual" cards, older men across cultures describe a kind of visual "hunger" in the figures—the young man and woman are staring at something; or the rope climber is climbing so as to get a better view, to see something beautiful. For younger men visual activity is not an end in itself, but has a guidance and feed-back function.

Realism (Autonomous)

2. *Innurturance:* Emphasis on an arid environment, hostile to life and to human effort.

Passive Mastery

Anxious Constriction

3. *Perceptual Restriction:* Emphasis on a few accurately perceived details; but avoidance of total, integrated scene.

Syntonic Passivity

4. *Fantasied Relief from Innurturance:* Description of arid desert and suggestion that background clouds might bring rain.

5. *Integrated Denial of Innurturance:* Rain actually falls from distant clouds; or foreground gully forms the banks of a body of water.

Magical Mastery

Denial

6. *Gross Perceptual Denial:* Arbitrary introduction of helpful, fertile or nurturant agents: angels, churches, flowers, money, sheets of water.

Projection

7. *Uncontrolled Natural Forces:* Floods, marine volcanoes, sea battles, sinking ships, drowning, or dangerous animals.

TABLE IV—The "Desert Scene": distribution of stories by age, culture, and theme

		35-49		50-59		60+	
Active mastery A. *Coping* 1. Human agency	Navajo	5	13*	3	5	2	2
	Lowland Maya	5		1		—	
	Highland Maya	3		1		—	
B. *Realism* 2. Innurturance	Navajo	7	9	1	3	1	1
	Lowland Maya	—		1		—	
	Highland Maya	2		1		—	
Passive mastery A. *Anxious constriction* 3. Constriction	Navajo	4	6	5	7	10	13
	Lowland Maya	—		1		1	
	Highland Maya	2		1		2	
B. *Syntonic passivity* 4. Fantasied relief from innurturance	Navajo	5	7	7	8	9	9
	Lowland Maya	2		—		—	
	Highland Maya	—		1		—	
5. Integrated denial of innurturance	Navajo	5	10	8	10	5	6
	Lowland Maya	3		2		1	
	Highland Maya	2		—		—	

Magical mastery							
A. *Denial*	Navajo	1	7	2	16	11	31
6. Gross perceptual denial	Lowland Maya	—		9		14	
	Highland Maya	6		5		6	
B. *Projection*	Navajo	1	8	2	3	6	12
7. Uncontrolled natural forces	Lowland Maya	6		1		6	
	Highland Maya	1		—		—	
			60		52		74

Navajo N = 100
Lowland Maya N = 53
Highland Maya N = 33
TOTAL N = 186

* Chi Square = 38.297, DF = 12, P < .001

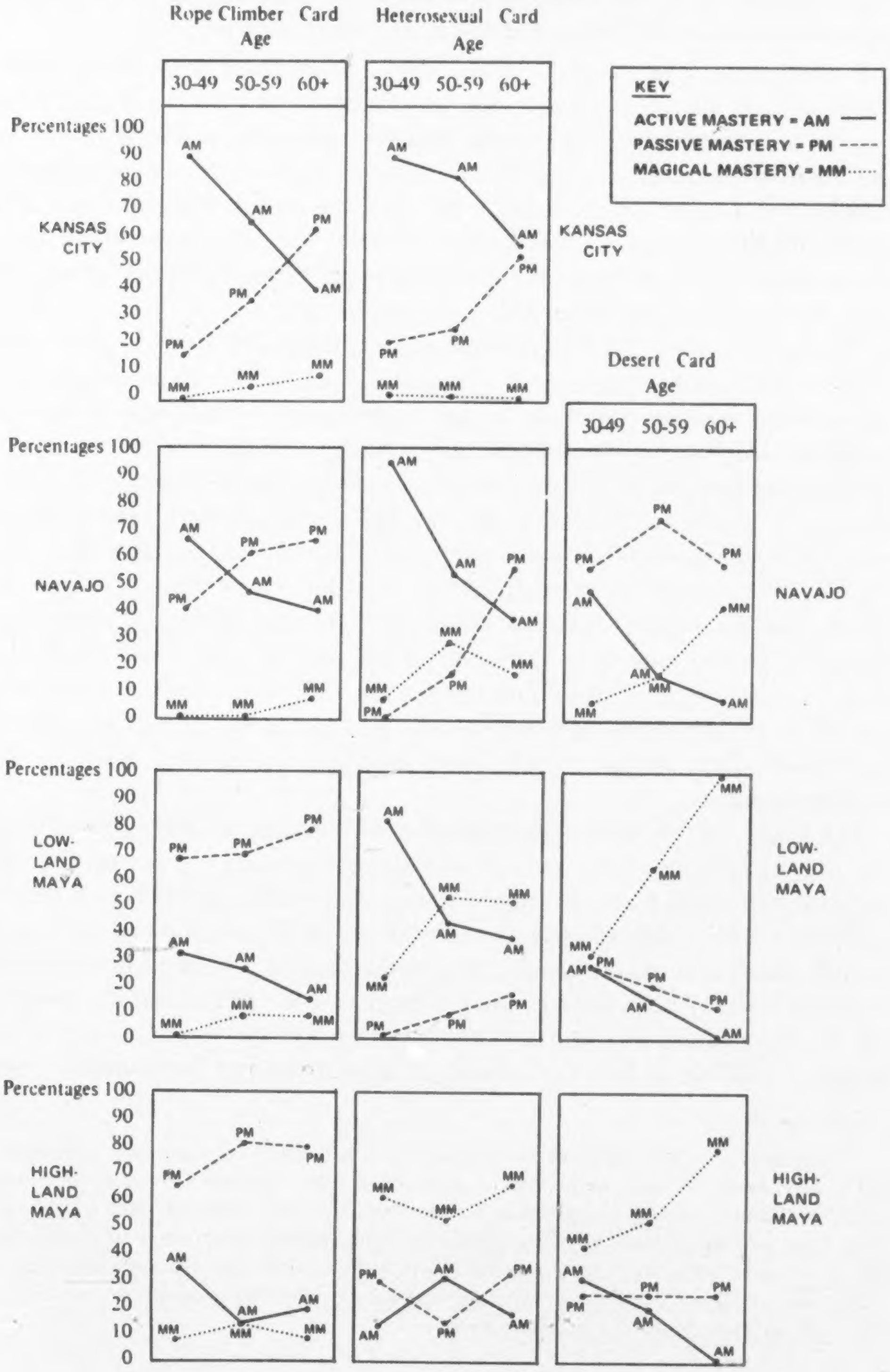
Table IV shows a clear age progression in the data, defined especially by categories 1, 2, 4, 5, and 6, away from recognition of the painful card issues and towards various perceptual metaphors of denial. Forty-eight percent of responses given by all men in the youngest group clusters in categories 1 and 2, indicating the relative readiness of this group to look squarely at unpleasant issues. By contrast, 62 percent of responses given by 50-year-olds are found in 5 and 6, the two perceptual denial categories, as are 75 percent of the responses from men aged 60 and over.

Accordingly, the data generated by this card support the prediction concerning the age-grading of Magical Mastery: with advancing age it appears that the boundary between wish and reality may break down, so that one's wishes regarding nature are legislated into a consoling picture of it. The way things "ought to be" becomes in later life a design for "the way things are."

Table V shows the distribution of each mastery orientation expressed in percentage terms by age groups, by cultures, and by the previously discussed cards. As regards age distributions, the table indicates that the mastery orientations, whatever their relative importance for any particular culture, tend to rise or decline with age as predicted. For example, Active Mastery is the primary Kansas City and secondary Lowland Maya orientation; yet, for both these cultures, Active Mastery declines with age. Similarly, Magical Mastery is relied on only minimally in Kansas City, but is an important Lowland Maya orientation; yet, for both these disparate cultures and across all cards, Magical Mastery increases with age. By the same token, there is an independent culture effect, in that — particularly if the two Mayan groups are treated as one unit — all four cards tend to

TABLE V—Distribution of mastery by card, by culture, and by age

From Gutmann, D., "The Country of Old Men: Cultural Studies in the Psychology of Later Life." *Occasional Papers in Gerontology*. No. 5, Institute of Gerontology. The University of Michigan-Wayne State University, April, 1969, pp. 1-37. Reprinted by permission of the Institute of Gerontology, The University of Michigan-Wayne State University.



agree on their rankings of cultures by mastery preferences: Kansas City is consistently highest on Active Mastery, for any card or age cohort, and lowest on the more passive or regressive styles. While Navajo has a more even mix of styles than Kansas City at any age, it consistently, across cards, ranks higher on Active Mastery and lower on the passive and regressive orientations than do either of the Mayan Indian groups.[4]

Furthermore, the "Desert Scene" card, by contrast with other cards, seems to shift all age groups, in all the subject cultures, towards the more passive and "regressive" end of the mastery spectrum, while maintaining the relative distinctions in terms of mastery preferences between them. Possibly, this card, which depicts the natural rather than the personal world, registers in these three Indian societies the preliterate world view towards nature. One can be effective with people; but nature is refractory, and one is either stoic about this or hopes for the best.

To sum up, when TAT data from other cultures are coded according to criteria developed in the course of analyzing urban U.S. data, the mastery orientations tend to distribute by age as predicted. While particular cultures can amplify or retard particular mastery orientations, these emerge and decline according to a schedule first discerned in the Kansas City data. Moreover, the mastery criteria can be applied in standard fashion across cultures with predictable consequences. This finding suggests that the mastery orientations are transculturally distributed and that they can have standard mental representation across societies that themselves maintain different conventions as to the form of the mental life. Thus, while the mastery orientations may require particular cultural conditions for their optimum development and expression, the potentials for each mastery orientation seem to have an intrinsic, and perhaps genetic, basis in the human organism.

We know that social environments are vital to the process of transforming species characteristics into human ego potentials; but it may also be the case that social forms are themselves an extension — even a creation — of those intrinsic ego capacities whose developed form they nurture and require. Man and society are the joint products of a process of interlocking evolution, where each system, the personal and the social, was the context for the maturation of the other. Once such a process is under way, it no longer is possible to find neat chains of dependent and independent variables. Man's nature created the social circumstances of his nurture; but that nature is itself intrinsically social in its orientation and character.

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[4] There is also something of an independent card effect, in that the "Heterosexual" card tends to pull more Active Mastery among younger Indians and more Magical Mastery among older Indians than does the rope climber card. This age-graded patterning of card responses matches independent observation of sexual behavior in these societies: younger Indians prove their manhood through sexual conquest; but older men try to ignore the whole issue precisely because of the rivalry and fighting that excite the younger men.

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DISCUSSION

Dr. Lambo, noting that the whole premise of the ego and its development is based on Freudian or neo-Freudian theory, wondered how valid it may be to extend such analysis to other cultures. He would like to see Kansas City compared with Chicago, or with any other culture within a group of national cultures, rather than across national boundaries.

Dr. Gutmann replied that, to take a Passive Mastery stance and quote authorities, he thought his description of Active Mastery resembled McClelland's (1961) description of need-achievement. Using the N Ach perspective across cultures, McClelland has obtained results that make sense in a comparative framework. One is not surprised, in other words, to find that certain groups score high on McClelland's need-achievement measures and that others have low scores. Independent measures and observations based on standards and conceptions indigenous to non-Western cultures will tend to confirm McClelland's rankings among these cultures, even though such rankings are based on a very "Western" conception, that of achievement and achievement motivation.

Dr. Gutmann thought that a similar point could be made concerning his ranking of cultures in terms of, for example, their predilection for Active Mastery. He went on to say that when the ego-mastery conceptions are applied to data generated in a variety of societies, the resulting age X mastery distributions replicate those found originally in the United States, where these conceptions were developed. Finally, Dr. Gutmann said, it is not a form of exploitation or colonialism to look at either cultures or individuals from a standpoint that they themselves do not recognize or use as they evaluate themselves. The only scientific questions are whether or not our results have some validity and whether they further our understanding of the cultures or individuals in question.

A SURVEY OF CULTURAL DIFFERENCES IN SEX ROLE TRAINING AND IDENTIFICATION

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METHODOLOGY

I shall concentrate here primarily on two aspects of what we mean by "sex role": socialization and work roles. One might define sex role here as referring to the expectations in a society for typically masculine and typically feminine behavior irrespective of sexual activity. "Identification" refers to the process by which children internalize the norms or virtues and values that are appropriate for their roles as defined by their societies.

I am presenting a cross-cultural paper using data that are somewhat unusual to those who are not familiar with this area. It is based largely on a review of studies reporting on customary behavior in a world-wide sample of societies. The basic data are ethnographic reports by anthropologists who have done field studies of these societies. Most of them utilize material that is classified topically in something called the Human Relations Area Files originally developed by George Murdock at Yale — a file that has classified ethnographic material by topics. The method used in the studies I am going to report is to have raters read anthropologists' descriptions of particular kinds of institutions, customary behaviors, and so on, in a society, and make judgments of these reports, which are then represented in numerical ratings.

We have variables like age of independence training or age of weaning, as well as severity of independence training, in which there was a descriptive scale going from extreme punishment to no punishment or mild punishment. The rater had actually transferred descriptive material into a numerical rating scale.

This method allows for the quantitative representation of culturally descriptive data, which in turn allows for statistical analysis of some relationships among ratings. I am therefore addressing myself to the question of whether there are pancultural similarities and differences in customary behavior that can be analyzed statistically.

The unusual feature of these data is that a society, rather than a single individual, is the unit of analysis, so that each datum or case represents a society. The behavior being reported, then, is the usual behavior common to a group of people who share a common culture rather than the idiosyncratic behavior of any single person. While this method obviously does not have the precision obtained by experiments or direct observation, it does have some advantage of stability of measurement inherent in the fact that one is really working with some kind of normative or modal measure.

The societies used in these studies are primarily nonliterate, nonindustrial folk or tribal societies of the world, such as the American Indian societies, people in the South Pacific, the tribal societies of Africa.

The reason for basing investigations on these societies is that only by doing so can one see the full range and variation of human customs and institutions. Certain customs important to this discussion, such as household composition, form of marriage, and initiation ceremonies, are usually coterminous with a particular society; hence there really is not much identification variation. Other customs, such as age of weaning or severity of punishment, may vary considerably from mother to mother within a society. However, a study of groups of families in a society that are deviant in some way does not yield the same results as would a study of normal variation across societies. If a mother is deviant, the effect upon her children may come about because she is doing something that is unusual for that culture rather than because of the practice in and of itself.

ECOLOGICAL INFLUENCES ON SEX ROLE TRAINING

The primary antecedent variable that I shall be describing is what anthropologists have called household composition. I am going to be talking about four types of family composition, two based on monogamous marriage and two on polygynous.

The household with which most of us are familiar is the monogamous nuclear household, consisting of a husband, wife, and their unmarried children of both sexes. Grandparents, aunts, uncles, and cousins live elsewhere in their own nuclear houses. In such societies some houses may include a widowed parent of either the husband or the wife, but rarely will two mothers of young children share the same domicile.

The monogamous extended household consists of an extension of a monogamous nuclear household along either patrilineal or matrilineal lines. In the patrilineal extended household, adult males remain in the home of their father, so the household in its simplest form consists of a husband and wife, their unmarried daughters, their married and unmarried sons, and the wives and children of all married sons. The sons stay put and the daughters move out. Large extended households may consist of two or even three generations of brothers and their descendants.

In matrilineal societies, the adult women remain at home with their parents. A similar extension occurs across the female line. From the point of view of socialization, children are raised in such households with grandparents, aunts, uncles, and cousins in the same house, as well as their siblings and parents (Murdock and Whiting, 1950).

In polygynous marriages there are two types of household structure. The first is called polygynous-polygynous, meaning a household in which the wives share the same house. This usually takes place in societies in which a man marries sisters who have been raised in the same house. If a man marries women who are unrelated to each other, each wife has her own house. The husband usually has a house of his own and divides his time among his wives. The household here — and this is quite an important one — is what anthropologists call a mother-child household, one in which the mother and her children live in the house and the husband lives somewhere else. They are obviously similar to the fatherless homes in our own society.

There are a few societies that practice polyandrous marriages, or the marriage of one woman to several husbands. These are so rare that it is difficult to get statistical results, so I have not included them in the analysis.

Although human beings show a remarkable ability to adapt a variety of social structures to a variety of environments, as we shall see, household type is not entirely independent of climate and environment.

There is some evidence that a role differentiation of parents — mothers versus fathers — is largely pancultural. Talcott Parsons suggested that men are the instrumental leaders of a family and women are what he called the "expressive leaders." The instrumental leader is the executive head of the family, the person who primarily organizes activities and is the final judge, arbitrator, and dispenser of punishment. The expressive leader is the family mediator and conciliator, the person who resolves hostilities, soothes over disputes, and is generally the peacemaker.

One of Parsons' colleagues, Dr. Zelditch (1960), found across a wide sample of societies that women in general took the expressive role and men the instrumental role.

SOCIALIZATION PRACTICES

Barry, Bacon, and Child, who have done several articles I shall be referring to, have found some pancultural similarities in socialization practices. Theoretically, I think the important aspect of their observations is that if one finds certain things common to men and women or the training of boys or girls that are present across a wide variety of societies, one wonders whether this is not somehow a behavioral sexual dimorphism. It may be that there are masculine and feminine aptitudes for learning certain kinds of behavior, and it is easier for the society to work with them than to work against them.

Barry, Bacon, and Child (1957) find that over 80 percent of the societies in their sample give more encouragement to boys than to girls for self-reliance and for achievement. Self-reliance is defined as taking care of one's self, as contrasted with responsibility for helping with the family work, and achievement is defined as striving for excellence in performance.

These authors find that 82 percent of their societies train girls to be be more nurturant, and 61 percent train them to be more responsible. That finding will become clearer when I report on some of my work.

Most societies, 62 percent, trained boys and girls to be equally obedient; but when there was a sex difference, 35 percent of the remaining 38 percent trained girls to be more obedient than boys. Unfortunately, these authors did not include aggression training in their analysis.

They also find that the size, but not the direction, of these sex differences varies with subsistence economy and the structure of the society. That is, while the kind of behaviors emphasized for boys and girls remains constant, the degrees of differential emphasis do not.

Large differences between the sex training of boys and girls are found in polygynous and extended families, while small differences occur in nuclear families. In a nuclear family the adults must be prepared to take over at least the essential work roles of a member of the opposite sex, because if either the husband or wife gets sick or is absent, unless there is a child old enough to do that work, who else is going to do it? In the extended family situation there is another adult, man or woman, somewhere around to take over.

In the economic realm, large sex differences occur where strength and skills are required to insure the food supply. This is true in nomadic societies, particularly those that hunt large animals, a task requiring both strength and skill, and in agricultural or herding societies that keep large animals, either as dairy herds or for the cultivation of grain crops. Small sex differences occur in societies that are heavily dependent on root crops, gardening of things like yams or potatoes, which women usually can do perfectly well and often do by hand. Where there are grain crops, large animals are used, and large animals are handled by men. Evidently the strength and stamina of men are needed to bring in the food in that kind of economy.

Barry, Bacon, and Child (1959) find a kind of over-all masculine-feminine difference in societies that seems to be important. Societies that are dependent upon animal husbandry particularly emphasize responsibility and obedience, or in other words somewhat feminine virtues. Hunting and gathering societies emphasize achievement and self-reliance. Hunting is in general a more skilled occupation than primitive agriculture, so the difference between a good and a bad hunter is greater than the difference between a good and a bad farmer.

SEX TYPING OF WORK ROLES

Baldridge and I have done a study on sex typing of work roles (unpublished). We began by listing the tasks reported in each society and then categorized them fairly broadly. We did not include tasks done only by specialists. We wanted to look at the work roles that were filled by typical men and women in a society. We found a good bit, again, of fairly pancultural sex typing. Chores that are typically done by men are those that require skill and self-reliance.

The argument I am making here is that high self-reliance and high achievement of boys are reflected panculturally in the work assignments of adult men, assignments like hunting, trapping, deep-water fishing, warfare, politics, care of large animals, clearing and plowing, and major building. Typically, women's work included household tasks, child-care work, nursing the sick, and something called cosmetics. We also found that women typically do food gathering. In a hunting and gathering society, the men do the hunting and women do the gathering of vegetable matter. In a fishing society, the women do the kind of shore fishing that seems rather like gathering—getting small shellfish and that sort of thing—whereas the men go out for deep-sea fishing.

Women also typically carry loads. I think this should be explained. In nomadic societies, when they are on the move, it is a little like what was reported among the baboons: the women do most of the carrying, because the men are carrying their weapons in a ring around the women and children to protect them.

The finding that boys are encouraged in high achievement is substantiated by these data. In most societies, boys and men are expected to perform more highly-skilled chores. We have rated each of the chores on skill level and found that the mean skill level required for men was higher than that for women in almost all societies. Male tasks also are those that require strength and involve risks.

Barry, Bacon, and Child, as I have said, found that girls were trained to be more responsible than boys. This seems a little nonsensical in view of the fact that men are the leaders of a society. But we find that this is reflected in the work roles, which seems to account for this result.

Because boys' chores are more highly skilled in general, and because some of them may require strength, boys are more likely to be introduced to a greater number of their work roles at adolescence, when they are getting their secondary sex characteristic strength and when they are old enough to concentrate on their work, while girls are introduced to their work roles earlier. Some girls typically learn a greater proportion of what they are going to have to do as adults earlier than boys do.

Furthermore, we found that typically girls and women did more things. In English we have a term "Jack of all trades." I decided it really should be "Jill of all trades," because within any society women do a wider variety of things. Their tasks were not as tied to the economy, so that whereas men would be either hunters or agriculturists or fishermen, women were doing household tasks and taking care of the children and this sort of thing.

Across societies, there is more variation in men's tasks; that is, women are taking care of children and doing housework wherever they are. But within a society, women do a wide variety of tasks, though at a lower level of skill. I think these findings throw considerable light on the Barry, Bacon, and Child finding that responsibility training is usually stronger for girls than it is for boys.

We also found that men's chores were more likely to be co-operative, requiring group effort. This may be one of the reasons why men are trained for higher achievement. If they start doing a poor performance, they are going to foul up somebody else's work. Women, on the other hand, do things that typically they do by themselves; they can work either better or worse or slower or faster.

We have found some data that are interesting for identification: sex typing of the work assignments extends to the teaching of these skills. Mothers are the most frequent teachers, particularly for young children. The mothers usually teach the girls, which means they usually teach the low-skill tasks. Fathers teach older boys in high-skill tasks. As a result, we find that girls are almost never taught the chores typically done by adult men; nor are they ever required to do work that is typically done by adult men. But boys may be taught by their mothers to perform tasks that will be done by adult women.

There is thus more discontinuity in the training of boys. I think you can see why. They are there around the house with the women. They may not yet be introduced to their adult work roles, because they tend to learn them later. Meanwhile the women put them to work, I think probably even more if there is not a younger sister.

IDENTIFICATION

Ever since Freud proposed his theory of the Oedipus complex. psychologists have been concerned with the problem of how children learn to incorporate the values of their parents and internalize the roles of their appropriate sex. Most modern psychologists take the position that identification stems primarily from an early dependence of babies on their mothers, which provides a kind of biological basis for love and the need to maintain the affection and approval of a powerful and beloved person. According to this view, children internalize the moral standards of their parents and learn to imitate adult behavior, because in doing so they evoke parental praise, whereas failure to do so evokes displeasure and punishment.

Within this general view, theories of identification can be divided into those that emphasize the role of parental love, praise, and reward in evoking identification with the nurturer and those that emphasize the role of parental displeasure, scolding, and punishment.

John Whiting (1959a) has attempted to some extent to put those two together. He argues that children identify with the mother because she has the expressive leader role, primarily out of love; that they identify with the father more in terms of what Freud would call identification with the aggressor. But the unifying concept that he proposes is that the child identifies with the parent whose status he envies—that is, the person whom he sees as the most powerful, whether nurturant or non-nurturant.

Whiting also argues that the type of superego that comes primarily from maternal and paternal identification varies. He is talking about the male and female superego. Freud talks about this, and Whiting gets there from a somewhat different route.

Whiting's evidence for the distinction between male and female standards of morality is based on the sources previously described: the Parsonian distinction between the feminine role of expressive leader and the masculine role of instrumental leader, and the evidence presented by Barry, Bacon, and Child that girls are trained to be nurturant, while boys are trained to be self-reliant and achievement-oriented. He assumes that nurturance training is incompatible with punitive behavior, while concern for high standards and independence of performance is supportive of rigidity of norms and their stern enforcement.

We come now to the first problem, I think, that makes identification somewhat more difficult for boys. Since infants and young children are cared for by women in practically all societies, they start out identifying with their mothers. Girls need only maintain this initial identificaton to learn approprate sex-role behavior, whereas boys face the necessity of shifting their identification from their mother to their father and to adult male roles. This identification process is apparently easier for girls than it is for boys in virtually all societies, the most important reason being the shift necessary for boys.

But the work-role requirements are also easier. Girls are introduced to their low-skill tasks earlier than boys are to their tasks. They are taught these tasks by the appropriate role model—their mother. They are typically assigned chores done regularly by women, whereas boys also may be assigned chores that are done regularly by women. The conditions for learning sex-appropriate behavior are therefore easy for girls compared to the learning conditions often faced by boys.

So far we have talked about those differences in identification that appear to be pancultural—if not universal, at least independent of variations in household compositions. But the degree to which identification is easy or difficult, at least for boys, appears to be affected by this family structure.

Whiting argues that children in mother-child households will identify particularly strongly with their mothers, because in such households they are the persons who control the children's access to resources. This conclusion can also be reached on the assumption that identification is a matter of high dependency. This early period of initial dependency in mother-child households is usually ensured by strict observance of prolonged post-partum sex taboos—that is, a prohibition against intercourse by the mother for a period following the birth of the child.

Almost all societies have this for a period of a month or so, but a number of societies have it for a period of two years or as long as four years. The societies with the long post-partum sex taboo tend to be mother-child household societies. They also tend to occur in rainy, tropical climates, in areas where staple foods are low in protein, where there are high rates of kwashiorkor, which is a severe protein deficiency among children. In these societies, the mother's milk is the safest and most nutritious food for young children, and the taboo is usually rationalized or explained in such societies as a way of ensuring that the child will not have to be prematurely weaned.

These societies tend to be polygynous. Polygyny in part, then, seems to be an adaptation to this kind of danger for the children and a cultural adjustment to the survival value of prolonged nursing and the subsequent necessity for prolonged sexual abstinence.

The mother-child societies apparently find it necessary to institutionalize special procedures that seem to be designed to sever the strong emotional tie between sons and their mothers and shift the boy's identification to men and masculine role behavior. The institutionalized procedure that has been studied in this connection is initiation ceremonies, particularly initiation ceremonies involving circumcision, which is usually done at adolescence rather than in infancy as with Jews. Women are excluded from the ceremony. Boys are isolated from the society of women after the ceremony and typically do not go back to live in their mothers' houses, so there is a shift in residence. Such ceremonies presumably serve to break the boys' dependency on their mothers and establish their identification with men.

In contrast, early identification of boys with fathers is apparently made maximally easy in the nuclear household where the father is around, where there are only two adults. Whiting argues that the father is seen as a rival consumer of goods in such households, has high status, and is therefore envied by his children. We find that independence training is earliest, and achievement training is highest and punishment of aggression least severe in these nuclear households, so they tend to train for specifically masculine kinds of virtues, high self-reliance and independence. In these households parents apparently do not need to punish aggression to suppress it, probably because the boys are identifying with their fathers and their fathers' moral standards fairly early.

Mother-child households have the longest period of dependency and the lowest achievement training, with extended families and polygynous-polygynous households have an intermediate position.

Extended families, which have been neglected in the cross-cultural identification literature, are the families with high sex differences in training. Extended families in some ways are the easiest in both sexes.

The socializing patterns of nuclear versus mother-child households appear to be maximally different, but in extended families we find essentially that boys are trained to male virtues, girls are trained in female virtues, but there is not a high degree of emphasis on either one or the other. Since there is a work force of both adult males and females in extended families, it is the system that most allows people to "do their own thing." Therefore these families tend not to emphasize high achievement, probably because highly-skilled performance is not necessary where substitute workers are readily available.

On the other hand, what is absolutely essential to extended families is peaceful cooperation among the family members. They have to be able to get along with each other. So we find suppression of aggression is the strongest in these families.

The nuclear-family societies tend not to have initiation ceremonies for boys. They apparently do not need them. In the extended-family societies, half of them do and half of them do not have initiation ceremonies, but when they do, the ceremonies tend to be nonpainful ones like the Bar Mitzvah or the Hindu sacred thread ceremony.

This analysis assumes that initiation ceremonies are a mechanism for emphasizing identification with sex roles. The data indicate that the identification of boys is influenced by the type of household in which they are raised. What about girls? Analysis of Minturn's unpublished data indicated that initiation ceremonies of girls are not influenced by household type. The reason for this is readily apparent: mothers are ubiquitous. The type of household determines the extent to which men are around the children, but women are always present and caring for small children in any type of household.

The antecedents of female initiation ceremonies are, then, essentially different from those of male ceremonies. They are also simpler and clearer, probably because identification for girls is simpler and clearer than it is for boys.

The most complete study of female initiation ceremonies has been done by Judith Brown (1963). Dr. Brown finds three cultural conditions antecedent to female initiations. Combined, these three antecedents account for virtually all the societies in her sample.

First, Brown argues that in many societies the shift in residence of women at marriage serves as an initiation rite, in that it severs the dependency ties of girls with their families of origin in the same way that shift of residence after initiation does for boys. On this assumption she predicts and finds that female initiations are most likely to occur in matrilocal societies—that is, societies in which girls do not shift residence at marriage. In these societies female initiation serves the function served by marriage in societies where marriage entails a shift of residence.

Secondly, Brown finds that female initiation ceremonies occur in societies where the work performed by women is particularly important to the subsistence of the society. She argues that in such societies the initiation ceremonies serve to emphasize the importance of responsible performance of the feminine role. This finding again substantiates the relative importance of responsibility training for women.

Finally, there are a small number of societies who practice painful initiation, often involving a genital operation. These societies seem to be imitating similar rites for adolescent boys. They are typically patrilocal societies with long post-partum sex taboos, where circumcision rites for boys are practiced.

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DISCUSSION

Responding to a question from Dr. Neugarten about the classification of cultural activities in work roles in societies in which the manufacture of cultural artifacts takes on a high salience, Dr. Minturn said that for lack of time she had omitted a list of activities that she and Dr. Baldridge had found were not sex-typed. One of them was handcraft. That means that in some societies the men may do this work and in others women do it. Therefore there was not any pancultural effect.

"Wouldn't that whole set of data bear upon your notions of skill and achievement?" asked Dr. Neugarten. Dr. Minturn said it would, but that even with handcraft the men were doing the high-skilled craft. "It surprised me, but it is true."

Dr. Thomae asked whether Dr. Minturn had meant to include industrial societies when she had said that identification is more difficult for boys. Dr. Minturn said she had not. "In child development," Dr. Thomae noted, "we see that it is more difficult for girls, especially between the ages of six and ten, because girls of that age choose masculine toys. They have to adjust."

"Industrial societies can get quite complicated," Dr. Minturn said, "because of the variety of work roles open to both sexes. I have been talking about societies in which almost all adult men would be following one kind of occupation."

In view of all that had been said about sex differences in this conference, Dr. Money cautioned against drifting into the assumption that all little human beings are identical. There has been a great tendency to do that in social, psychological, and anthropological work over the last quarter century, he thought. Dr. Minturn said she was not assuming that.

Dr. Money also urged the giving of more thought to an idea he had mentioned in his presentation: that girls do not just learn the girl role and boys the boy role, but that they learn both of them and then suppress one. This is an important possibility that he thought had not received proper attention.

Her data, Dr. Minturn said, suggested that boys have to do that more than do girls.

ATTITUDES TOWARD SEX VIEWED CROSS-CULTURALLY

Psychologists have increasingly used the concept of critical periods for understanding the development of human behavior in the early years, said Dr. Ambrose. Although the concept has been plagiarized and misused, there is conviction on the part of some people that there is a similarity between the findings of behavioral scientists, on the one hand, and biologists and embryologists, on the other, that exposure to certain kinds of input of certain ages has an optimum effect, whereas exposure at other ages has very little effect. Dr. Ambrose therefore asked the two speakers on sexual behavior to say whether they have any indication from their work as to whether exposure of infants to their parents' sexual activities is likely to have different effects on the infants if they experience it at different ages.

Remarking that he thought it likely that the question of a child's seeing his parents having sexual intercourse may be a somewhat traumatic matter for Westerners, probably because of psychodynamic thinking and some of Freud's ideas, Dr. Caudill said it would have more to do with the feelings of the parents than with those of the child. If he could take the reports of his thirty Japanese mothers seriously, it would appear that they wait until they believe the child to be sound asleep. From statistics on sexual behavior published in Japan, it seems that this decreases the length of time and the frequency of sexual activity. He doubted that this had much effect on the child. He did not know how much a child—starting at the age of three to four months and going up to ten or twelve years of age—actually sees his parents engage in intercourse. He imagined it would be more frequent than in Western cultures because of the sleeping arrangements.

There is also the matter of bathing, Dr. Caudill said. The child will bathe with a cross-sexed parent or with the same-sex parent frequently throughout life, but especially up to the ages of ten or twelve. In Japan at golf courses and other public places, there are large share baths like small swimming pools. The men are, of course, naked, but there is an etiquette to it: one carries a small towel, which is used as a wash cloth, to hide the genitals as one goes into the bathing place. Though today there is less shared bathing than there used to be, there still is a fair amount of it. A Westerner who lives in Japan for some time learns to block out, as the Japanese almost completely block out, any sexual reference to such behavior. It really is not sexual, except to a person from another culture, who might have his feelings aroused by that sort of situation.

Dr. Kagan said he thought Freud's error was to assume that the child would feel as anxious as he would. He believed that to a child who grows up in the living arrangements of the Japanese, watching his parents having intercourse is a normal activity and not necessarily traumatic.

What Dr. Caudill had said about Japan, Dr. Lambo believed to be true about most African cultures. The exposure is continuous right from childhood. There is not a great deal of emotional attachment to what is seen. It is not supposed to be something that he should not see, so there is no attachment of guilt to it. It is also part of the fact that throughout the culture, even in adult life, most people, certainly in the traditional cultures, wear almost nothing.

In Western societies, Dr. Lambo suggested, there is much more flexibility in the lower classes of the population. There is not a great deal of guilt attached to seeing mommy and daddy having sexual relations. It may be true that the higher one goes on the social scale, the greater the amount of guilt or emotional attachment to things one is not supposed to see. Therefore the social sanctions come about.

Dr. Money reported that among the Australian aborigines of Elcho Island in Arnhem Land, who have, since World War II, come from their nomadic life to settle in a small village of about 1,000 people, the custom is for the children to play at sexual intercourse. They usually do not see their own parents, although there would be no restriction against their doing so, since all the people sleep in a little hollow in the sand around the camp fire. Chiefly the younger children see the slightly older children engage in intercourse, and spying on teen-agers is a favorite sport at the football oval on Saturday nights. Boys in the preschool in a very unselfconscious way may make pelvic thrusting movements, especially at nap time. The teacher pays the same attention to it as to thumbsucking and it does not constitute a special problem. The response of the adults to their children's sexual behavior around the camp fire is to laugh. That is the end of it.

When the boys have their big circumcision ceremony around the age of eight, Dr. Money continued, one of the obligations from then on is that they are not to be public about their sexual play. All sorts of interesting complications enter at this point, because the totemic avoidance relationships become obligatory after the age of eight, making it difficult for boys and girls to play at sexual intercourse. So when the girls play at games of house, one of them will use a stick as a penis so that she can play the father. This has nothing to do with homosexuality. It has to do with preparedness for sexual intercourse.

Dr. Money's interpretation of this situation was that it is essential in the culture to have a completely open attitude toward sexual intercourse because it is still a polygamous culture in which the man with whom the adolescent girl will go to live is already decided before the girl is conceived. The man may be as old as thirty, forty, or even fifty years. He may have two, three, four, or five wives. The girl may regard the sexual relationship as perfunctory and sometimes downright unpleasant. But she is able to perform it because of all the rehearsal she has had in childhood.

Though supporting in principle what Dr. Kagan had said, Dr. Jensen added that, because of their attitudes toward sex, middle-class Americans seem to want to conceal from their children everything they do that is related to sex—even kissing each other. Freud's idea that it is traumatic for a child to witness his parents having sexual intercourse is probably generally valid, Dr. Jensen thought. However, there is also other sexual behavior of parents that is "traumatic." For example, if parents are kissing and their child asks them what they are doing, the parents are likely to answer, "We aren't doing anything," with the further implication: "Go away and forget about it." The child gets the message that sex is wrong and something his parents dare not talk about. His attitudes toward sex are similarly shaped. Parents of any social class who accept sexual behavior in a natural way and explain it without projecting their shame or guilt will not make the experience traumatic; they can make it a healthy experience for the child. The child's response to parents' sexual behavior depends largely on how the parents regard it.

In the world at large, Dr. Minturn said, the American family arrangement in which the baby is put to sleep in a room by himself is not the rule. It is largely a product of our affluent society. In most parts of the world people live in one room per family. Therefore it is undoubtedly common in most societies for the children to see their parent engage in intercourse. The same thing is true for birth. In an Indian village she had seen a group of children, including the little son of the midwife, standing around watching a delivery. Such procedure is certainly much less frightening than the mysterious process of taking the mother to the hospital where the child cannot go to see her and from which she comes home bringing an ugly little thing that then takes all her time and attention.

Sleeping arrangements in the United States not only are unlike those in many parts of the world today, but are also unlike what was customary in Europe up to the seventeenth or eighteenth centuries, when laws were passed forbidding children to sleep with their parents, evidently for fear the mother would roll over and suffocate the child, Dr. Caudill said. In his data on Japanese families, whether there is one or more bedrooms, if anyone sleeps alone it is likely to be the infant up to one year of age (but only about one in ten such infants sleep alone). After one year of age even these infants are brought into the family sleeping arrangement, which means the children sleeping in the room with both parents or the parents separated with one or more children sleeping with each of the parents.

Remarking that sex is a biological instinct—something that has to happen if the species is to be maintained—Dr. Caudill said that he was interested, as an anthropologist, in knowing what happens to that universal biological urge in the process of its cultural molding. He believed that there is a somewhat different phrasing, by the time of adolescence and adulthood, of sexual behavior and desire, say, in a middle-class American compared with middle-class Japanese. Up to now, he has considered that sexual desire in middle-class America is heightened and focused upon as something desirable, but also something with some guilt encompassed in it. It has also become blended psychodynamically with aggression. To have good sexual intercourse, one must be aggressive about it. Someone has to take the initiative. Both partners have to be somewhat aggressive. He thought that the focus and emphasis on sex that one sees in America is not so great in Japan, with that country's greater stress on the dependence or interdependence of people in contrast to the greater stress in America on the independence of the individual. Such experiences as that of having been interdependent with somebody, particularly an older woman, ever since one was born get in the way of being able to be directly sexual. Therefore, though the Japanese are obviously sexual, since they reproduce themselves, sexual behavior does not have the meaning that is focused on it in our culture. There is a different phrasing of an essentially biological activity that is hard to disentangle from the broader matrix of the culture with its alternative avenues to pleasure that are brought into combination with sex as such.

We have few data on this subject, Dr. Caudill continued. Kinsey's data are chiefly distributional—how often and how long do people have intercourse—data that do not answer the questions about the meaning and context of sexual behavior in different cultures.

Dr. Caudill asked what we mean by sex: just intercourse, or all the other things that have highly sexually-tinged aspects? Sex in any culture is a private matter when you get down to intercourse. But there is always an appropriate context or vehicle for the behavior that has to be institutionalized. In that sense, behavior in Japanese bars is quite sexual in the sense of petting and dancing and holding hands, etc. And it is public to the extent that it occurs in an appropriate place in a group of people. Before World War II it was considered inexcusable in Japan to hold hands or kiss in public. Now it is quite commonplace. There has been a shift in the precursors to direct sexual behavior in Japan today.

In the interest of greater explicitness, Dr. Garn suggested that the subject was not sex, but attitudes toward sex. "We are really talking about talking about sex," he said. He asked Dr. Lambo whether he found it an interesting, important, and basically scientific question to be discussing attitudes toward sex rather than sex itself. Viewing the discussion from the point of view of another culture, did he think that the group members were appearing to be climbing out of Victorian England by their finger nails, titillating themselves as they do so, or making sense?

Dr. Lambo replied that, from the point of view of behavioral science as a whole, the group could legitimately go into scientific exploration of attitudes toward sex, but he thought that much of what is called sex in Western culture is actually symbolic rather than real. Much is made of the issues of sex that are not sexual at all, whereas in other cultures there is a small nucleus that Westerners in fact attribute to sex. Everything, movements, etc., is seen in a sexual context. Everything is sexualized. Dr. Lambo thought that this is a global matter.

Man is inevitably beset with anxiety throughout his life and for generations, Dr. Kagan said. He needs a palliative. In earlier centuries the palliative was religion. In the twentieth century it is sex. Perhaps it will be drugs in the twenty-first. Since man is always uncertain and anxious, perhaps that is why our culture appears to be so eroticized.

Dr. Hirschhorn took issue with Dr. Kagan on this point. "Perhaps psychology is the new religion," he said. "The question of sex really was as much an answer to anxieties in ancient Greece and ancient Rome as it is in our society today. Pictorial and written evidence of this fact is quite strong. Emphasis on sex has not changed much in Western society in 3,000 years. I think, as Dr. Garn suggested, that what we are doing is talking differently about it. We are trying to use scientific methods to analyze what we are talking about when we discuss sex."

The question of therapy in psychiatric disorders having a great deal to do with sex seemed to Dr. Lambo to be pertinent here. He recalled that in the 1950's when he was studing psychiatry in England he came into contact for the first time with the long-standing kind of sexual psychopaths. Dr. Maxwell Jones was in charge of a place for aggressive psychopaths, most of whom were suffering from a sexual disorder. After Dr. Lambo had spent about a year in that hospital, when some of the patients were about to be discharged, he had asked the director what were the criteria for discharge. The answer was that the man in question now had good insight into his sexual problem and had no more guilt about his homosexuality. He could now function as a homosexual without guilt.

Dr. Ambrose, referring back to the critical period concept, suggested that homosexuality may well prove to be a good example in the human being of an irreversible outcome of some early developmental deviation. Part of the therapy may be just helping the patient to accept the irreversibility.

Dr. Jost asked the psychologists in the group to say how much in the attitude toward sex derives from intercourse and how much from menstrual bleeding. How much in the whole attitude is a matter of concealing sex or sexual relations and also menstruation? Dr Kagan said he thought there was a connection between the two, but knew of no data on the subject. The question of attitude toward menstruation in African cultures was referred to Dr. Lambo.

"This is where the cross-cultural approach is so important," Dr. Lambo replied. "Most women in Africa guard their menstrual period because they feel that the discharge is part of their entire bodies, an extension of their bodies. It is a valuable body product, like the finger nails. If this part of nature is got hold of, they may be in great danger. It has nothing to do with sex at all."

"So there is more psychological differentiation," suggested Dr. Kagan, to which Dr. Lambo assented.

Dr. Tomae spoke of a study by Stevens published in this country using the material of fifty-seven societies on the lengths of the taboos around the menses and the post-partum period as an index of the oedipus complex. He found a high correlation of the length of the taboos and the severity about them. Length of this time was correlated also with the severity of child-rearing practices. That means that a variation went across several cultures, not mainly Western cultures.

Menstrual taboos and fear of menstrual blood, Dr. Minturn said, occur in societies that have the long period of mother-son dependency. It is not correlated with over-all severity of socialization, though it is correlated with severity of sex training specifically, so it does seem to be a product of inhibitions in the particular area.

As for the question of whether Western society is erotic, Dr. Minturn added stress on the difference between sex and talking about sex. She suggested that we are indeed, as Dr. Garn had said, climbing out of the Victorian age in a kind of prudish, pornographic fashion. An example is the enormous amount of money that has been made out of the Playboy Clubs, which are safer than a chaperoned dance at a girls' finishing school in terms of actual involvement. They are not like the Japanese bars; a man cannot date the bunny.

Dr. Ambrose asked Dr. Caudill whether, in attempting to understand the factors that affect the development of sexual patterns in older children and adults, we should look not only at the previous sexual experiences of the child, but also at quite different functions that have to do with very early contact between the mother and baby, contacts that the psychoanalyst might call a libidinal sexual contact with no overt sexual function.

"The Japanese child obviously has a great deal more physical contact with his mother than does the American middle-class child," Dr. Caudill replied. "I asked my mothers, when their oldest child—my index child—was two and a half years old: 'Do you think that your child knows whether he is a boy or a girl?' About two-thirds of the Japanese mothers sort of pooh-poohed the question, saying that the child did not know this. Almost all of the American mothers said yes, the child did know. Obviously the American children are constantly told by their mothers that they are a boy or a girl, and partly as a consequence of this, boys and girls begin to behave in quite different ways."

The difference in sex role, Dr. Caudill continued, is noted earlier and more forcibly in American than in Japanese culture. Therefore he thought that as the child gets older this fact makes a good deal of difference in his approach to the problem of what we usually mean by "sexual behavior." It seems to be a gentler thing in Japan—more of an appetite like that for food or the desire to be warm in winter or the desire for sleep. There also are differences in familial interpersonal relations that influence attitudes toward sexual behavior in the two cultures. In America, the husband and wife reserve a part of their lives separate from that of their children, whereas in Japan the father virtually gives up his wife and she focuses most of her attention on the children. There is less competition in Japan between the father and children for the attention of the mother. This close mother-child tie in Japan endures in almost its childhood form well into adulthood. With regard to fantasies involving mothers, the mother is a big problem for an adult to handle psychologically anywhere in the world, but even more so in Japan. The encapsulated image of his mother doing things for him is so strong in the mind of the Japanese man that it gets in the way of his having what we in the West call a good direct heterosexual relationship with another woman when both he and she are adults. He rather wants the woman to do things for him. He tends to be more passive in the relationship than the Western male is supposed to be. In a theoretical sense, these differences probably make a difference in the resolution of the oedipal situation in the two cultures.

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